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ERRATA

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P. 432, line 12, omit comma after "users"

P. 477. This paper by G. T. NIGHTINGALE and R. B. FARNHAM is a paper of the journal series of the New Jersey Agricultural Experiment Station.

THE
BOTANICAL GAZETTE

September 1935

STUDIES IN THE PHYLOGENY OF THE BETULACEAE[1]

I. FLORAL AND INFLORESCENCE ANATOMY AND MORPHOLOGY

ERNST C ABBE

(WITH TWO HUNDRED NINETY-EIGHT FIGURES)

Introduction

The vexed question of the disposition of the Amentiferae in the phylogenetic classifications of today has led to this study of the Betulaceae. It is especially significant, in view of the importance attached to floral and inflorescence characters in present-day phylogenetic studies, to attempt to clarify the concept of the ament as it exists in this family. While much may be learned in this connection from a comparison of external structures alone, it is desirable to supplement this by a comparative study of the vascular anatomy of the florets and of the inflorescences, since it has been convincingly demonstrated for other groups of plants that the vascular system usually responds but slowly to changes in the external form of the floral organs.

But it is felt that, helpful as it may be in clarifying the morphology of the organ under consideration, a comparative study of the vascular systems of reproductive organs alone does not provide a sufficiently broad foundation for indicating interrelationships between groups of plants. In an attempt to supply this extra evidence for the Betulaceae, a study of the secondary xylem anatomy

[1] Contribution from the Laboratory of Plant Morphology, Harvard University.

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of many of the species has been made, the precipitation reactions have been studied (6), and the observations of others in the fields of chromosome number and behavior and pollen morphology have been evaluated. These supplementary fields of evidence will be considered in a later paper.

It is felt that by establishing working hypotheses for the interpretation of the evidence from each of these fields, and then combining the results on the basis of the multiple working hypothesis (5), conclusions can be reached which would provide a basis for evaluating the phylogenetic positon of the Betulaceae. While the need of experimental verification of hypotheses and conclusions is strongly felt, the means of supplying this lack are not yet clear. Furthermore, a firm foundation should first be laid in comparative morphology before such means of verification are put into practice.

Methods and materials

Herbarium material, when used, has been softened by immersion for several days in water kept warm on a warming table. A great majority of the studies are based on freshly collected specimens preserved in 70 per cent ethyl alcohol, a developmental series from several individuals of each species often being studied. Preparatory to study, a catkin is transferred to distilled water, allowed to soften, and with the usual instruments carefully dissected under water in a dish lined with beeswax. A pair of iridectomy scissors has been found helpful for the more delicate work. The better dissections have been preserved by mounting on slides in MASSART'S medium (18), while the best were drawn and later sectioned. Specimens were run up into paraffin (m.p. 56°–58° C.) through a slightly modified N-butyl alcohol series (38). Prior to this it was found necessary to soften all pistillate material with commercial hydrofluoric acid, full strength, for varying periods of time depending on the extent of sclerenchymatization encountered. Complete serial sections, 10–12μ thick, were made by means of a Spencer rotary microtome. The sections were stained on the slide with crystal violet and erythrosin (17).

While most of the specimens have been collected personally at the Arnold Arboretum, Harvard University, valuable aid has been

provided by Dr. E. ANDERSON, Dr. R. H. WOODWORTH, and DR. C. KOBUSKI of Harvard University. Except for the collections made by the latter two, these are authenticated by herbarium specimens. Material has also been obtained from Kew Gardens, England, on two occasions, and in the second case is identified by herbarium specimens. Further collections have been made by the writer on the north shore of the Gulf of St. Lawrence, in northern British Columbia and Alberta, in the Kaumajet and Torngat regions of Labrador, in North Carolina, in Norway, and in the vicinity of Ithaca, New York. Specimens have also been supplied from northern Canada by the late Dr. M. O. MALTE, from Greenland by Dr. M. P. PORSILD, from the state of Washington by Dr. W. C. MUENSCHER, from southern Labrador by Dr. H. F. LEWIS, and from China by Dr. A. N. STEWARD. Species which could not be obtained fresh were studied as described, from herbarium specimens kindly supplied by the herbaria at the Arnold Arboretum of Harvard University, at the Botany Department of Cornell University, and at the Botanical Garden, Berlin-Dahlem.

Identification of the material is based on the herbarium specimens or from field determinations. For checking the identifications, the writer is much indebted to Professor ALFRED REHDER of the Arnold Arboretum. The species names used are thus in general those to be found in his Manual of Cultivated Trees and Shrubs (25). Sets of herbarium specimens representing the material are to be found at the Arnold Arboretum and at Cornell University. The terms for the various subdivisions of the family and of its genera are in accord with the usage of WINKLER (35).

Terminology

The condensed nature of the units of which the aments in this family are composed makes it difficult to assign them to any of the more general categories of inflorescence types. The extreme shortness of the pedicels of the individual florets brings these florets together so closely that they appear to form a diminutive cyme, or cymule. This latter term has been adopted, with the reservation that basically these lateral members of the inflorescence may be racemose since they are racemosely arranged on the ament axis.

The bracts attached to the primary axis of the ament are referred to as primary bracts (B_1) throughout; in their axils occur the secondary axes bearing secondary bracts (B_2); and in the axils of these occur tertiary axes bearing tertiary bracts (B_3). The secondary and tertiary axes are considered to terminate in secondary and tertiary florets respectively (fig. 7). Since the members of the floral envelope, the tepals, are not differentiated from one another, they are referred to collectively as the perigon. It is felt, for reasons which will be taken up in another place, that the pistils of the Betuleae (*Betula* and *Alnus*) are reduced inferior ovaries, and therefore the term floret will be applied to these as it is to their homologues in the Coryleae (*Carpinus, Ostrya, Corylus, Ostryopsis*).

Cymule morphology and anatomy

It is clear from the anatomy of the cymules of the 64 species, varieties, and hybrids examined that the simplest basis for description is the type of anatomical system associated with a unilacunar gap system. It is true that the foliage leaves are trilacunar (28), but there is a simple transition from the trilacunar foliage leaves to the unilacunar bracts of the inflorescence. In figures 1-4 an attempt is made to show the relationship of some of the simpler types of vascularization in the cymules of the Betulaceae to the more common conditions in vascular systems. In figure 1 the simplest condition is shown, where the gaps left by all of the bract traces close after giving rise from their sides to the two branch traces which later coalesce to form the siphonostelic supply to the floral pedicels. In figure 2 is shown the result of failure of the gaps of the secondary and tertiary bracts to close and of the branch traces to coalesce, so that there is a dissected siphonostele in the pedicels. With concrescence of the bracts to their axillary branches the bract traces depart tardily from the bases of their respective gaps; and, if there is superimposed on this a shortening of the internodes, the bract traces are brought into a more intimate relationship with each other (fig. 3). This latter condition is closely approximated in proliferated pistillate aments to be described under *Alnus crispa*. When this system comes under the influence of lateral and dorsiventral pressures, comparable to those in the aments of Betulaceae, then a side
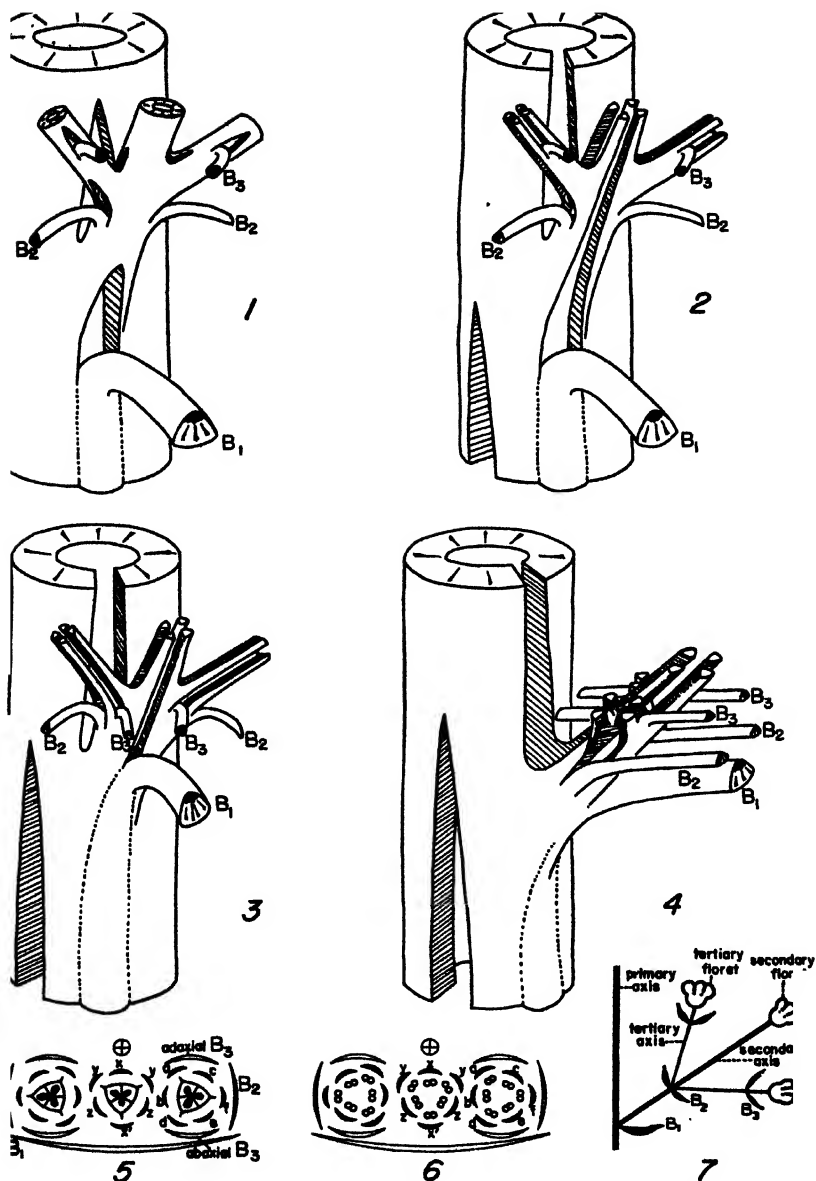
FIGS. 1–7.—Figs. 1–4, vascular system with unilacunar gaps in cymule at successive stages of reduction: 1, vascular system in which the gaps close; 2, same in which the gaps persist; 3, same in which concrescence and shortening of internodes modifies relative positions of bract bundles; 4, lateral view of vascular system shown in previous figure under the influence of dorsiventral and lateral pressures. Figs. 5–7, diagrams to show relationship of parts and terminology employed in the cymule: 5, floral diagram of pistillate cymule with trimerous florets; 6, same of staminate cymule; 7, schematic diagram of cymule.

view of the vascular system would appear as shown in figure 4. Here the courses of the vascular bundles to the bracts become nearly parallel and the pedicellar supplies of the individual florets appear to diverge dorsally from this aggregate of bract traces. A careful investigation of figure 4, however, will show that the traces to the individual florets take their origin from the sides of gaps left in the dissected vascular cylinders by their respective subtending bract bundles. Conditions closely comparable to this exist in the typical cymule in *A. crispa*. Throughout the family the vascular system found in the cymules of all species is patterned after that in *A. crispa*.

PISTILLATE CYMULES: MORPHOLOGY AND ANATOMY

Alnus, pistillate cymules

The pistillate catkin in the species studied[2] throughout the genus is composed of a great number of cymules arranged helically on the primary ament axis. In most of the cymules (except those at the distal end of the ament which are much reduced) there is a primary bract, the secondary bracts, and the abaxial[3] tertiary bracts. Very rarely an adaxial tertiary bract develops, but is generally much reduced (fig. 64). Tertiary florets are uniformly present, but the secondary floret commonly develops only in proliferations. One occurrence of quaternary florets has been found, but a consideration of this is reserved for the next paper of this series.

Because of the presence of a secondary floret in certain of the cymules of the proliferated material already mentioned, this makes a good starting point for a description of the vascular system of the cymule of the Betulaceae. Other than the presence of an occasional three-flowered cymule, this proliferated material of *Alnus crispa* differs from the typical condition in the elongation of the lower

[2] Section Alnobetula: *A. firma* var. *hirtella*, *A. crispa*, *A. crispa* var. *mollis*.
Section Gymnothyrsus: *A. subcordata*, *A. maritima*, *A. japonica*,
                      A. rugosa, *A. incana*, *A. hirsuta*, *A. rubra*,
                      A. tenuifolia, *A. jorullensis*, *A. spaethii*.
Section Clethropsis: *A. nitida*, *A. nepalensis*.
Section Cremastogyne: *A. cremastogyne*, *A. lanata*.

[3] The terms abaxial and adaxial are used in relation to the primary axis of the ament.
cf. floral diagram in figure 5.

internodes of the ament to such an extent that the ordinary dorsiventral pressure is removed. Thus there is a corresponding tendency toward a more normal orientation of the component parts of the cymules in this region. Above the region of marked proliferation the cymules have the morphology of those of ordinary catkins (fig. 40). A three-flowered cymule from the transition region is shown in figure 18 and a two-flowered one in figure 22.

The vascular systems of two- (fig. 20) and three-flowered (fig. 19) cymules show striking resemblances to those constructed on a purely theoretical basis in figures 1-4. Especially is this so for the three-flowered cymule. Starting well down in the primary axis of the ament (fig. 8), the first evidence of a vascular supply to the three-flowered cymule under consideration (cf. fig. 19 for reconstruction of the vascular system) appears as a wedge of xylem demarcated from the rest of the vascular system of the catkin axis by rather strong rays on either side. This wedge of vascular tissue soon departs from the main siphonostele, leaving a gap in the latter, and divides into three parts (fig. 9). The central one continues without further complications into the primary bract, while the two lateral bundles form arcs (fig. 10) which coalesce loosely to form the vascular cylinder with a gap in the region of the tardily departing primary bract of the secondary axis. No sooner is the cylinder of the secondary axis formed than the traces to the secondary bracts differentiate (fig. 11) and swing out and down (fig. 10), leaving a gap on either side of the vascular cylinder of the secondary axis. From the sides of these gaps depart a series of bundles, which bifurcate successively, one branch passing to the tertiary floret and the other branch remaining to supply the secondary floret. Thus bundles a, b, c, d, and e pass off laterally to form the vascular system of the tertiary axis and pedicel, while their homologues in the secondary axis fuse in pairs to form four of the traces (y, y, and z, z, fig. 19) in the pentamerous dictyostele. While this departure of the bundles to supply the tertiary axis is going on, the bundle to the abaxial tertiary bract also departs, leaving a gap from the sides of which are derived the bundles d and e. In addition to the homologues of the traces of the tertiary florets, there is another bundle in the secondary axis which is unique (x, fig. 19).
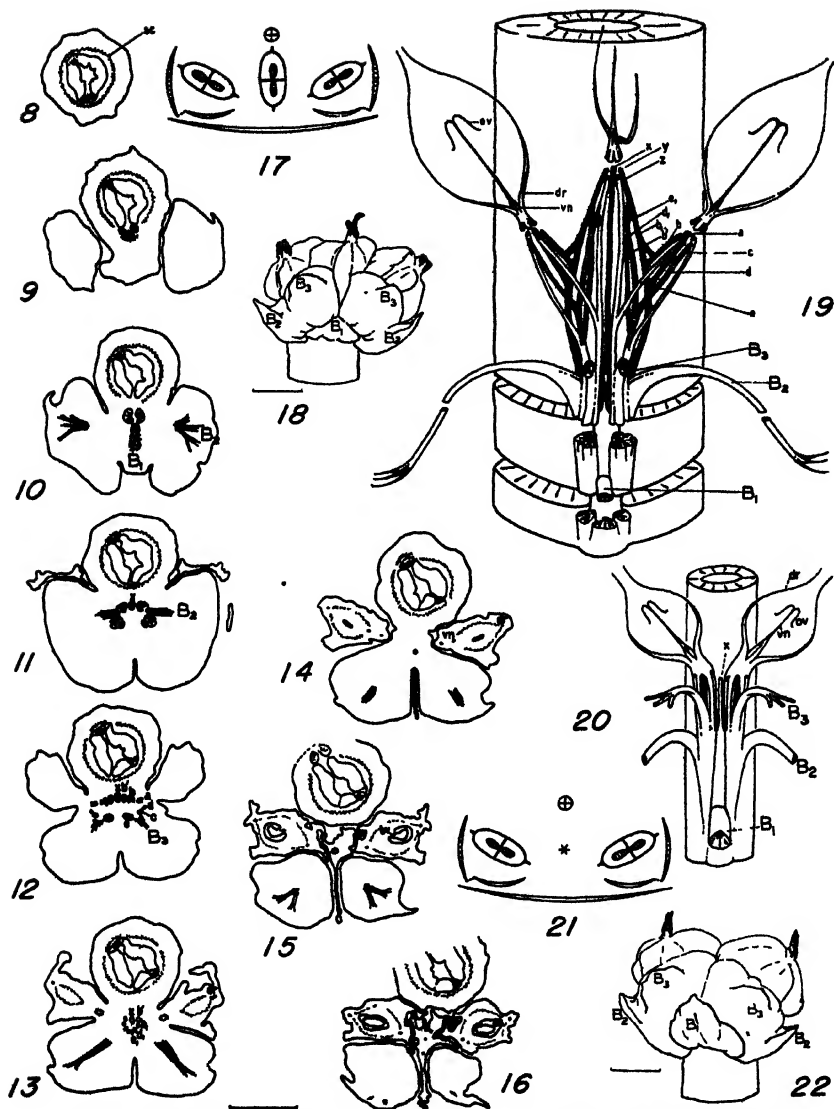
FIGS. 8–22.*—*Alnus crispa*, pistillate. Figs. 8–16, successive transverse sections through cymule shown in fig. 18; fig. 17, floral diagram of cymule shown in following figure; fig. 18, three-flowered cymule from base of proliferated ament; fig. 19, reconstruction of vascular system of cymule shown in preceding figure; fig. 20, same of cymule shown in fig. 22; fig. 21, floral diagram of cymule shown in following figure; fig. 22, two-flowered cymule from base of proliferated ament.

* Serial sections so arranged that they proceed distally through cymule. The scale of the figures is shown by a horizontal line near them representing 1 mm. Reconstructions are not drawn to scale and are sometimes exaggerated in the pedicellar region to show distribution of vascular tissue to better advantage. Pubescence and glands omitted in most cases from habit sketches. Single index letters used for vascular bundles or for the organs which they supply are in accord with usage indicated in floral diagrams, figs. 5 and 6. Bundles to bracts indicated by the abbreviations for primary (B_1), secondary (B_2), and tertiary (B_3) bracts. Other abbreviations used for structures found associated with the cymules are: *anas*, anastomosis; *dr*, dorsal trace; *invol*, involucre; *lam*, lamina; *lc* or *loc*, loculus; *ov*, ovule trace; *pd*, pedicel or pedicellar vascular system; *pg*, perigon; *sc*, sclerenchyma; *st*, stamen; *stip*, stipule; *vn*, ventral ovary trace.

The vascular system (fig. 20) of the two-flowered cymules (fig. 22) is essentially similar to that already described, except that in the absence of the secondary floret there is a corresponding absence of vascular tissue in the secondary axis, except for bundle x, which sometimes remains independent. Ordinarily x swings to one side or the other and fuses with one of the nearby traces, a behavior characteristic of the vascular supply to degenerate or recently lost organs.

The condition just described provides a convenient transitional stage to that of the vascular system (fig. 37) of a typical cymule in a variety of this species (*A. crispa* var. *mollis*). Figure 40 represents the external morphology of a cymule with the two florets removed, while figure 37 shows a reconstruction of the vascular system of such a cymule. The vascular system originates as a small number of protoxylem elements swinging out through the vascular cylinder of the primary axis and forming an elongate gap (fig. 23). As the xylem of the bundle progresses farther through the vascular cylinder of the primary axis, the gap becomes broader and the bundle accumulates more secondary xylem (fig. 24). By a lateral overarching, this bundle becomes roughly cylindrical in the cortex of the ament axis (fig. 25). At this level it represents the vascular cylinder of the secondary axis accompanied by the trace to the primary bract. The primary bract bundle soon becomes free (fig. 26) and, except for temporary anastomoses, continues independently into the primary bract. On either side of this primary bract trace there differentiates a large bundle, triangular in section (fig. 26), which splits to form two (fig. 28). The outer of these is the bundle to the secondary bract and the inner the bundle to the tertiary bract. The small traces (a, b, c, d, e) departing from the flanks of these large bundles correspond to the same traces described in the proliferated material (fig. 19), and are actually derived from the sides of the gaps left by the departure of the large bract bundles. Thus they represent the vascular system of the tertiary axes. So short a distance do they traverse before their reorganization in the bases of the florets, however, that this reorganization has already begun on one side of the pedicel while the bundles are still arising on the other side (figs. 29, 30). The trace x, which was first met in the proliferated material, is present as a weak bundle which later coalesces with the nearby trace e.
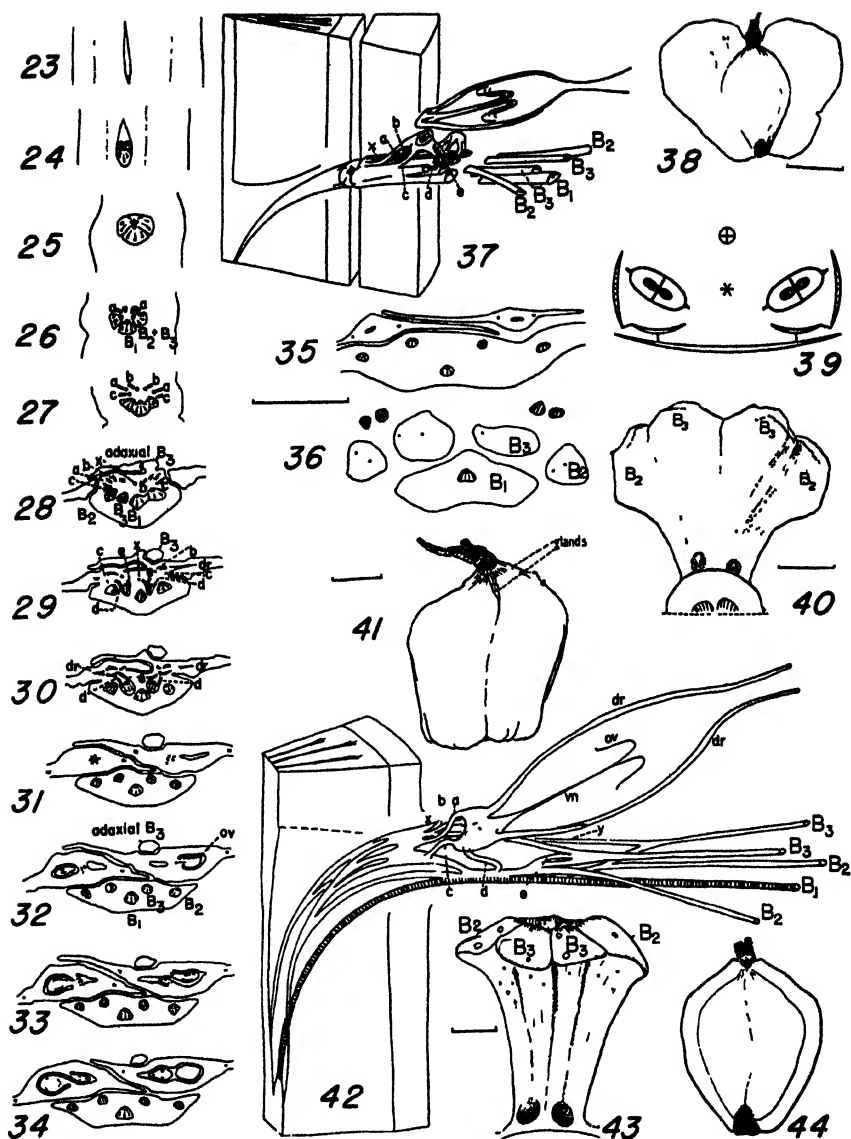
FIGS 23–44.—Figs. 23–40, *Alnus crispa* var. *mollis*, pistillate: figs.23–36, successive transverse sections through cymule similar to that shown in fig. 40; fig 37, reconstruction of vascular system of cymule shown in preceding figures; fig. 38, single floret shown from side of attachment; fig. 39, floral diagram of representative cymule; fig 40, cymule with florets removed. Fig 41, *A. subcordata*, single floret to show glands indicative of vestigial perigon. Figs. 42–44, *A. nitida*, pistillate: fig. 42, reconstruction of course of primary xylem in vascular system of cymule; fig. 43, cymule with florets removed; fig. 44, single floret viewed from side of attachment.

Interestingly enough, there was found in the example chosen for
sectioning a small, free, flattened structure opposite one of the
tertiary florets. This is probably the adaxial tertiary bract.

An essentially identical distribution of vascular tissue exists in the
other species studied in the section Alnobetula.

Representative of the section Gymnothyrsus is *Alnus incana*
(figs. 45–63). The morphology of the cymule (figs. 45, 46) is es-
sentially the same as that in the preceding section of the genus. In
the vascularization of the cymules (fig. 47), however, there are
some slight differences. More notable among these is the tripartite
nature of the origin of the vascular supply to a cymule (fig. 48).
This approaches much more closely to the vascular condition in the
proliferated material of *A. crispa* than to the typical material of
that species. Each of the three bundles accumulates secondary
xylem in its passage outward (figs. 49, 50) into the cortex and there
forms a rough siphonostele (fig. 51). In the course of the close as-
sociation of the primary bract bundle with the adjacent vascular
tissue, the vascular supply to the tertiary bracts remains with that
to the primary bract rather than with that to the secondary, as in
A. crispa. A better defined trace x is present here than in the normal
cymules of the s.[4] Alnobetula. As a further novelty, the trace x
splits (fig. 58) and either half (y and y) swings downward to fuse
(fig. 59) with the tertiary bract bundles after they become free from
the primary bract bundle.

The relationships of the cymule bundles are essentially the same
as described in s. Alnobetula, except for the absence of the trace e,
simplifying the supply to the tertiary florets. There is clearly a
correlation between the absence of the trace e and the prolonged
association between the primary and tertiary bract bundles. As
a result the trace e is not liberated in time to make connections with
the vascular system of the tertiary axis. Instead it accompanies the
tertiary bract bundles in their fusion with the traces y and y (fig. 59).
In other species of the s. Gymnothyrsus this intermediate behavior
of the trace e is more pronounced, showing the intimate structural
similarity between the members of the two subgenera. Mention
has already been made of the presence of an adaxial tertiary bract

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[4] Throughout the following pages, section is abbreviated as s.; subsection as ss.
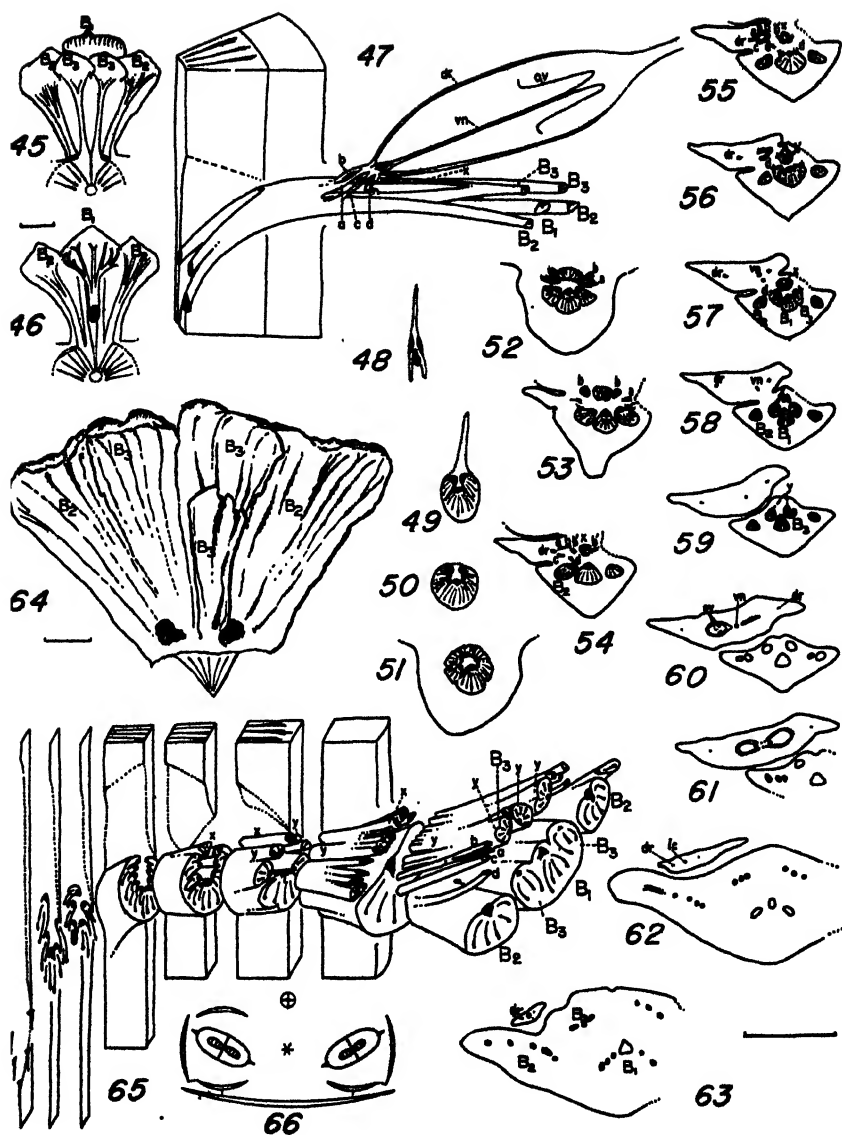
FIGS. 45–66.—Figs. 45–63, *Alnus incana*, pistillate: figs. 45, 46, upper and lower sides respectively of young cymule with florets removed and vascular system dissected out; fig. 47, reconstruction of vascular system shown in following figures; figs. 48–63, successive transverse sections of cymule similar to fig. 45. Figs. 64–66, *A. lanata*, pistillate: fig. 64, upper surface of mature cymule with florets removed (note presence of adaxial tertiary bract); fig. 65, reconstruction of vascular system of basal portion shown in preceding figure; fig. 66, floral diagram of cymule shown in fig. 64.

in *A. crispa* var. *mollis*. Examples of this condition were found also in *A. lanata* of s. Cremastogyne (fig. 64). The vascular system (fig. 65) of this cymule shows that the origin of the bundle to the adaxial tertiary bract is exactly as might be expected, since it is derived from the position corresponding to the origin of the abaxial tertiary bract, opposite. The bundles y and y on either side of the adaxial tertiary bract trace, the bundles a, b, c, d, and the abaxial tertiary bract trace, represent the vascular system of the tertiary axis. In this system the adaxial and the abaxial tertiary bract bundles occupy diametrically opposite positions, and lie in a plane at right angles to that of the departure of the bundle to the bract of the second order.

 In the species of s. Clethropsis, as shown for *A. nitida* (fig. 42), the vascular system originates as it does in *A. incana* (fig. 48), but the branch traces quickly subdivide so that there is a supply of at least six discrete bundles to the cymule, with the bundle x also often becoming separate. This characteristic is peculiar to the two species of this section and to the two species of s. Cremastogyne (fig. 65) which do not differ greatly in pistillate morphology and anatomy from those of s. Clethropsis. The origin of the tertiary vascular system is much the same as that in s. Gymnothyrsus. There is also a similar behavior of the trace e of this axis to that found in some species of s. Gymnothyrsus. Departing so tardily from the upper side of the tertiary bract trace that it cannot serve in the formation of the tertiary vascular axis, trace e fuses with the remnant of bundle y (fig. 42) at the time of the anastomosis of that bundle and the tertiary bract bundle. Of further interest in *A. nitida* is the sporadic occurrence of three-flowered cymules, their vascularization indicating that the supernumerary floret is indeed the secondary floret. These supernumerary florets occur in otherwise normal, non-proliferated aments.

In brief, the vascularization of the pistillate cymules of *Alnus* supports the generally current view that here the inflorescence was originally three-flowered (or possibly more) while the adaxial tertiary bracts have been lost by reduction. Furthermore, it is evident that the vascular system is not far removed from less modified conditions such as were discussed in relation to figures 1-4. Essentially,

figure 4 provides a schematic representation of the vascular system in most species of the genus. It is evident that the simple relationship of bract trace to branch traces is the basic feature in the organization of vascular system of the cymule in this genus.

Betula, pistillate cymules

The pistillate cymule of the species studied[5] in this genus generally has all three florets present, although only the secondary floret is present in some species of the ss. Nanae. The bracts of the third order are consistently absent, and among the species of ss. Acuminatae and ss. Nanae there is a tendency toward the loss of the secondary bracts.

There is great similarity in the origin and course of the vascular bundles supplying the cymules of all the species studied in the ss. Costatae. This may be attributed to two factors. One is the relative simplicity of the vascular supply contrasted with that of the cymules in *Alnus*, and the second is the presence of only primary xylem in the critical basal region of the cymule. This latter condition is doubtless correlated with the generally undifferentiated nature of the tissues in that region of the cymule, because of the dehiscence of the cymules from the primary axis of the inflorescence soon after the fruit matures.

As in many species of *Alnus*, the vascular system (fig. 67) of the cymule of *B. lenta* originates as three separate groups of primary xylem (fig. 68), the one at the base of the gap being the bract trace, the other two (the laterals) being the branch traces representing the

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[5] Section Eubetula

    Subsection Costatae: *B. schmidtii, B. globispica, B. lenta,*
                                  B. grossa, B. utilis var. *prattii,*
                                    B. lutea, B. medwediewi, B. nigra.

    Subsection Albae: *B. papyrifera* var. *subcordata,*
                                    B. papyrifera var. *occidentalis,*
                                    B. papyrifera var. *carpatica, B. coerulea-grandis,*
                                    B. pendula, B. japonica var. *mandshurica, B. populifolia, B. davurica.*

    Subsection Nanae: *B. michauxii, B. glandulosa, B. pumila, B. nana.*
  Section Betulaster
    Subsection Acuminatae: *B. luminifera, B. alnoides* var. *pyrifolia,* and *B. maximowicziana* (probably hybrid material).
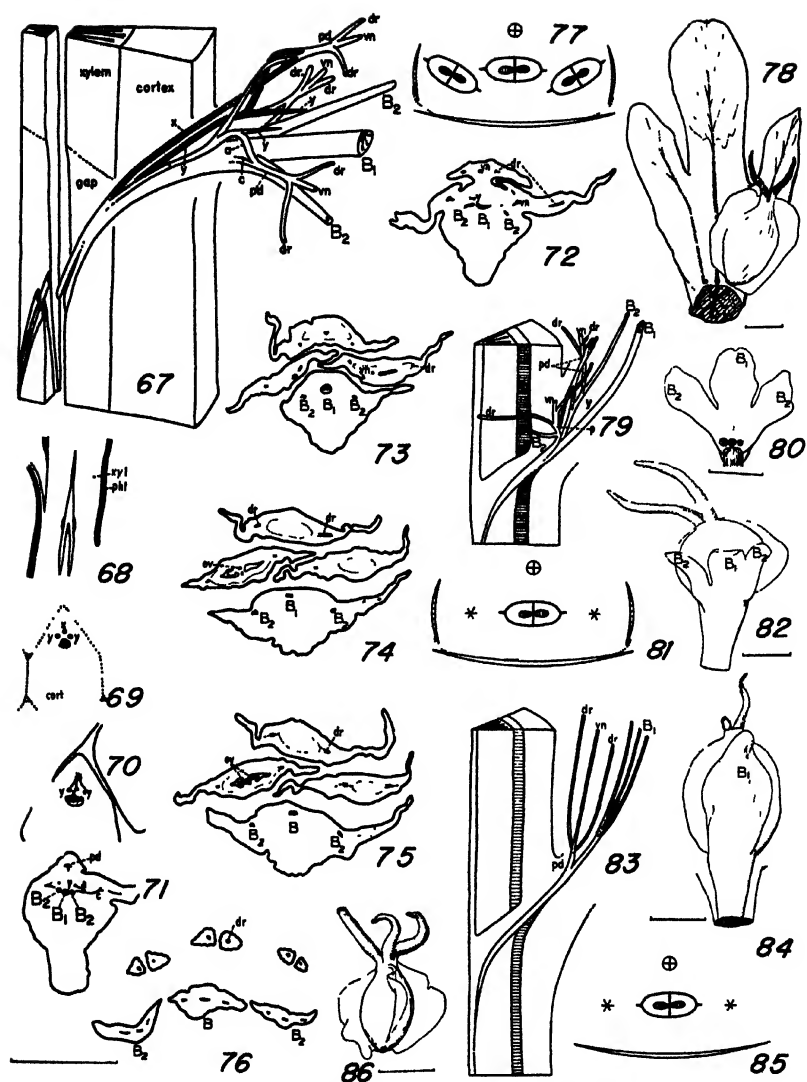
FIGS. 67–86.—Figs. 67–77, *Betula lenta*, pistillate: fig. 67, reconstruction of vascular system of cymule in section in following figures; figs. 68–76, successive transverse sections; fig. 77, floral diagram representative of preceding figures and of fig. 78. Fig. 78, *B. medwediewi*, pistillate cymule with two of the three florets removed. Figs. 79–82, *B. nana*, pistillate: fig. 79, reconstruction of vascular system of cymule similar to that shown in following figure; fig. 80, cymule with florets removed; fig. 81, floral diagram for cymule shown in following figure; fig. 82, one-flowered cymule seen from lower side of bract complex. Figs. 83–85, *B. michauxii*, pistillate: fig. 83, reconstruction of vascular system of cymule similar to that shown in following figure; fig. 84, mature cymule seen from lower side; fig. 85, floral diagram of cymule shown in preceding figure. Fig. 86, *B. luminifera*, tricarpellary ovary.

secondary axis. This is the vascular plan characterizing the incep-
tion of an axillary branch and its subtending leaf. A very weak
cylinder forms from these three bundles; in the cortex this breaks up
into four bundles (fig. 69). The uppermost of these four (x) con-
tinues into the secondary floret while the two lateral bundles (y and
y) bifurcate, each sending one branch to the secondary floret and the
other branch (a) to the nearest tertiary floret (figs. 69–71). In the
origin of the traces destined to vascularize the tertiary floret the
large lower median bundle gives off on either side a large lateral
branch from whose flanks small bundles (c and c) pass to the nearest
tertiary floret, to take part in the formation of the pedicellar supply
(fig. 71 *et seq.*). Ultimately these large lateral bundles form the
supply of the secondary bracts (fig. 76). After they contribute
vascular tissue to the tertiary florets, the traces y and y persist for
a short time, suggesting a possible tertiary bract supply.

Evidently there is a strong homology between the vascular sys-
tem of *Betula* and *Alnus*. Whereas in *Alnus* the poorest develop-
ment of the vascular supply to the tertiary floret still consisted of
four bundles (a, b, c, d), there are in ss. Costatae of the genus *Betula*
but two bundles (a and c), suggesting the formerly more complex
condition. The secondary floret in this section is somewhat more
richly supplied with vascular tissue (traces x, y, y) than are the
tertiary florets. It is significant that the trace x, which was found to
persist in *Alnus* when the secondary floret was absent, is also present
in *Betula* and constitutes a major portion of the supply to the
secondary floret. The other species of this subsection are essentially
similar to *B. lenta*, but tend toward a further simplification of the
vascular system, approaching that characteristic of the species in
the next subsection.

Instead of the three separate traces to the cymule characteristic
of most of the species of the preceding subsection, the species of
ss. Albae have but a single trace as the origin for the vascular sup-
plies of the primary bract and secondary axis. Apparently there
is such a close apposition of the branch traces to the intermediate
bract trace that, in the absence of secondary xylem, it is impracti-
cable to distinguish them. The trace x is found to be lacking after
the very weak cylinder formed earlier is broken up, and the second-

ary floret is supplied only by a branch from each of the traces y and y. A branch from each of the y's then passes to the nearest tertiary floret and fuses with trace c, which has meanwhile split off from the secondary bract supply. The remainder of each of the y's persists for but a short time. In all essentials the vascular system in the other species of ss. Albae is of this nature and represents a simplified version of the Costatae type.

Except for *B. michauxii* the species of ss. Nanae generally have three-flowered cymules, but in many cases entire aments may be composed of one-flowered cymules. In *B. michauxii* (fig. 84), not only are the cymules one-flowered but the secondary bracts are lacking, thus reducing the cymule to the ultimate in simplicity.

The vascular system of *B. nana* (fig. 79) exemplifies the condition in the three-flowered cymules of the species in this subsection. It is practically identical in vascularization with the cymules of ss. Albae, except for the absence of any prolongation of y beyond the departure of trace a, so that the two become indistinguishable. In the one-flowered cymules (fig. 82) of this species there is essentially this same vascular condition except that the tertiary florets and their vascular supplies fail to develop.

Finally in *B. michauxii* (fig. 84) no vascular supply (fig. 83) is even suggested for the tertiary florets or for the secondary bracts, while the single floret derives its vascular supply from a single branch of the single bundle to the primary bract. So simple is the inflorescence that the entire ament may be called a spike in the strictest sense of the term, although it is undoubtedly a spike by reduction, despite the fact that there are no vestigial traces to those parts of the cymules which are lost.

Of the species studied in ss. Acuminatae, *B. maximowicziana* stands as morphologically distinct, perhaps owing to the probable hybrid nature of the material. The vascular system is practically the same as that described for *B. lenta*, ss. Costatae. The other two species studied are of the greatest interest because they exhibit stages transitional to the apparent loss of the secondary bracts. In *B. luminifera* the cymules have the secondary bracts recognizable although reduced as are those shown for *B. alnoides* var. *pyrifolia* in figure 97. But in the latter there also occur more extreme conditions
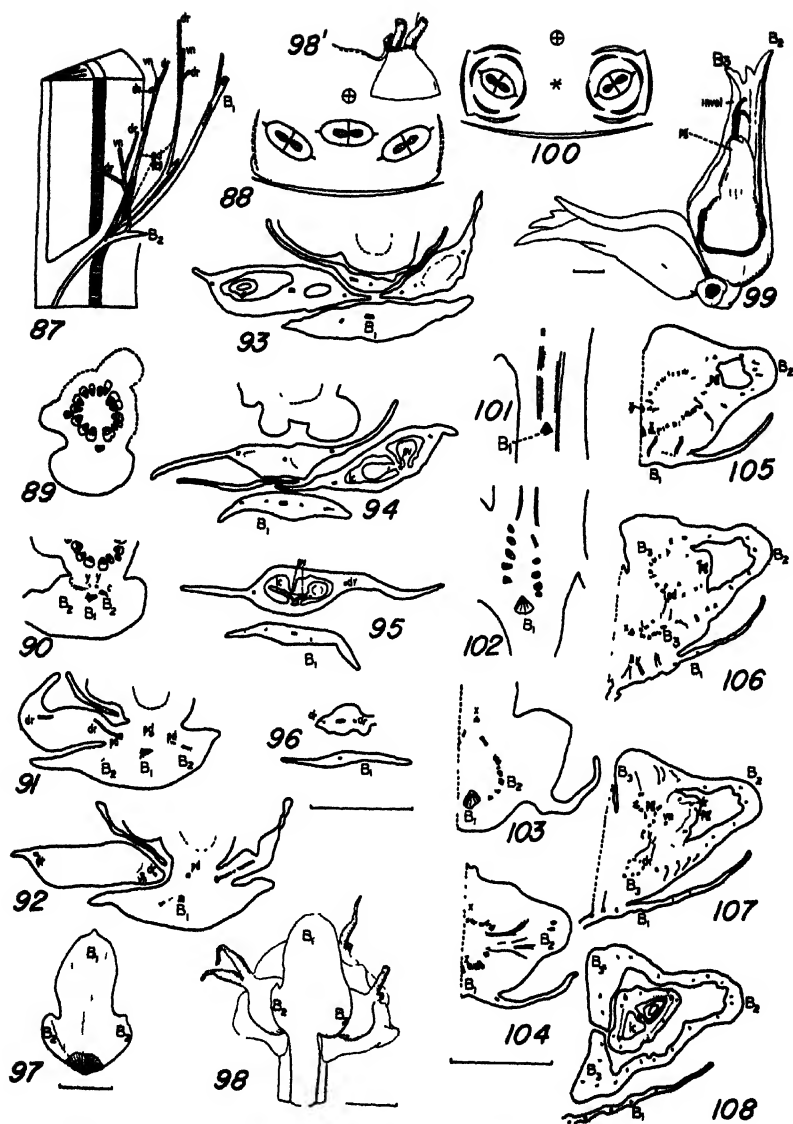
FIGS. 87–108 —Figs 87–96, *Betula luminifera*, pistillate· fig 87, reconstruction of vascular system of cymule; fig 88, floral diagram of cymule; figs. 89–96, successive transverse sections (*cf.* fig. 87). Figs. 97, 98, *B. alnoides* var. *pyrifolia*, abaxial and adaxial views of pistillate cymule. Fig. 98′, *B. coerulea-grandis*, upper portion of floret to show tepal gland. Figs. 99, 100, *Ostryopsis nobilis*, pistillate: fig. 99, cymule with portion of involucre cut away from around one floret; fig. 100, floral diagram of cymule. Figs. 101–108, *O. davidiana*, successive transverse sections through pistillate cymule similar to that shown in fig. 99.

(fig. 98), where one of the secondary bracts has merged completely with the primary bract while the other is still distinguishable. Not illustrated but commonly present in the material are cymules in which both secondary bracts are fused completely with the primary bract and only the distinctness of their vascular systems remains to indicate the basic nature of the cymule bract system. The vascular system of the cymules of *B. luminifera* (fig. 87) differs from that of the Albae in that the traces *y* and *y* do not take part in the vascular supply of the secondary florets, which is therefore composed only of trace *c*. The latter is derived from the weak secondary bract trace, which soon disappears.

The vascularization of the cymules in *Betula* provides no example of the presence of vascular tissue which may have supplied tertiary bracts. The secondary bracts are present in practically all the species, but they are so reduced in two of the species of ss. Acuminatae that a parallel series doubtless accounts for their absence in *B. michauxii* of ss. Nanae. While the secondary floret is always present in the cymules of the genus, the absence of the tertiary florets in *B. michauxii* is directly correlated with the extreme reduction of the cymule and its vascular system.

Carpinus, pistillate cymules

Perhaps the most striking feature in the inflorescence morphology of the species studied[6] is the disposition of the involucre, which in s. Eucarpinus is plane, three-lobed, or (if at the base of the ament) with either or both of the tertiary bracts free from the secondary one, the secondary bract much exceeding the tertiary ones in size. In s. Distegocarpus the adaxial tertiary bract is free, while the abaxial one is often so completely fused with the secondary bract that its identity is clearly detectable only in the vascular system of the cymule. Important also is the length of the internodes of the pistillate cymules of *Carpinus* which results in less telescoping of the vascular system, making it easier to interpret than that in the genera considered previously.

[6] Section Distegocarpus: *C. cordata, C. japonica.*
   Section Eucarpinus: *C. caroliniana, C. turczaninowii, C. laxiflora, C. orientalis, C. betulus.*
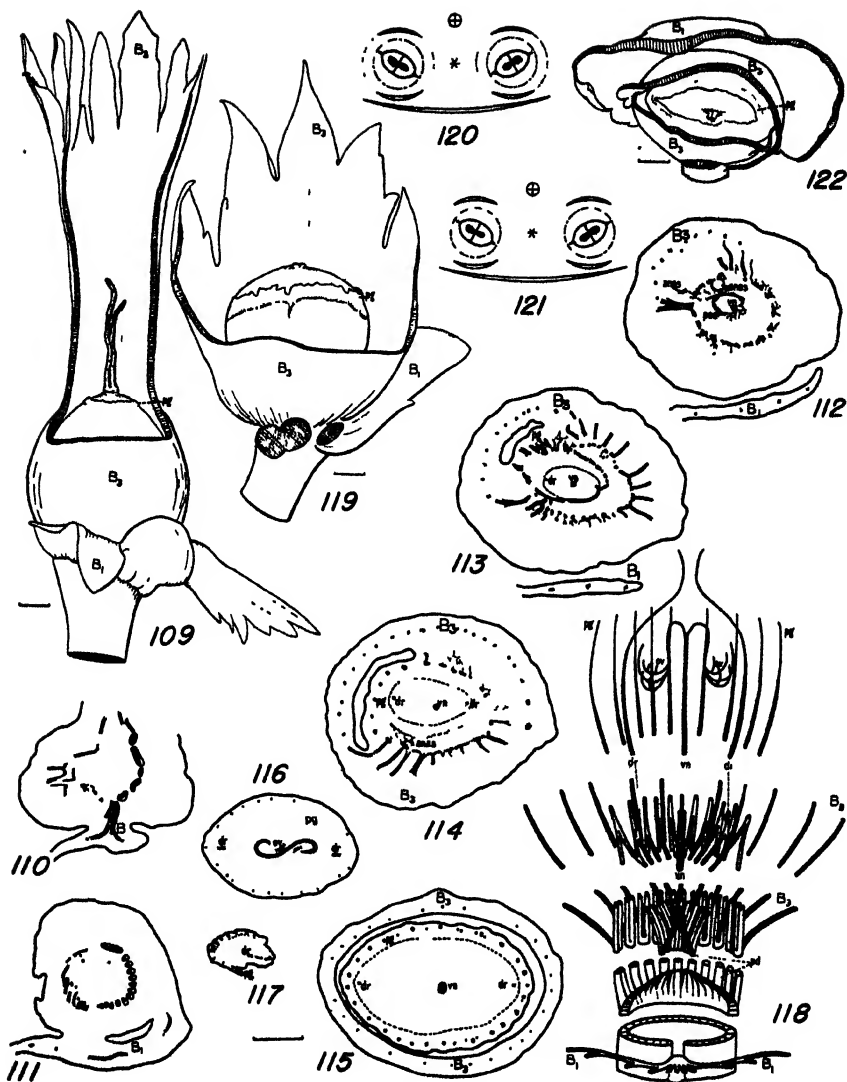
FIGS. 109–122.—Figs. 109–118, *Corylus cornuta*, pistillate: fig. 109, abaxial view of half-mature cymule with part of involucre cut away; figs. 110–117, successive transverse sections from base upward through cymule shown in fig. 109; fig. 118, reconstruction of vascular system of cymule shown in figs. 109–117. Figs. 119, 120, *C. maxima*, pistillate: fig. 119, lateral view of half-mature cymule, part of involucre cut away to show pistil, the other floret and cymules also removed; fig. 120, floral diagram of cymules shown in figs. 109 and 119. Figs. 121, 122, *C. heterophylla*, pistillate: fig. 121, floral diagram of cymule shown in following figure; fig. 122, view from above, smaller floret and other cymules of inflorescence having been removed as well as parts of involucral bracts to show upper portion of ovary.
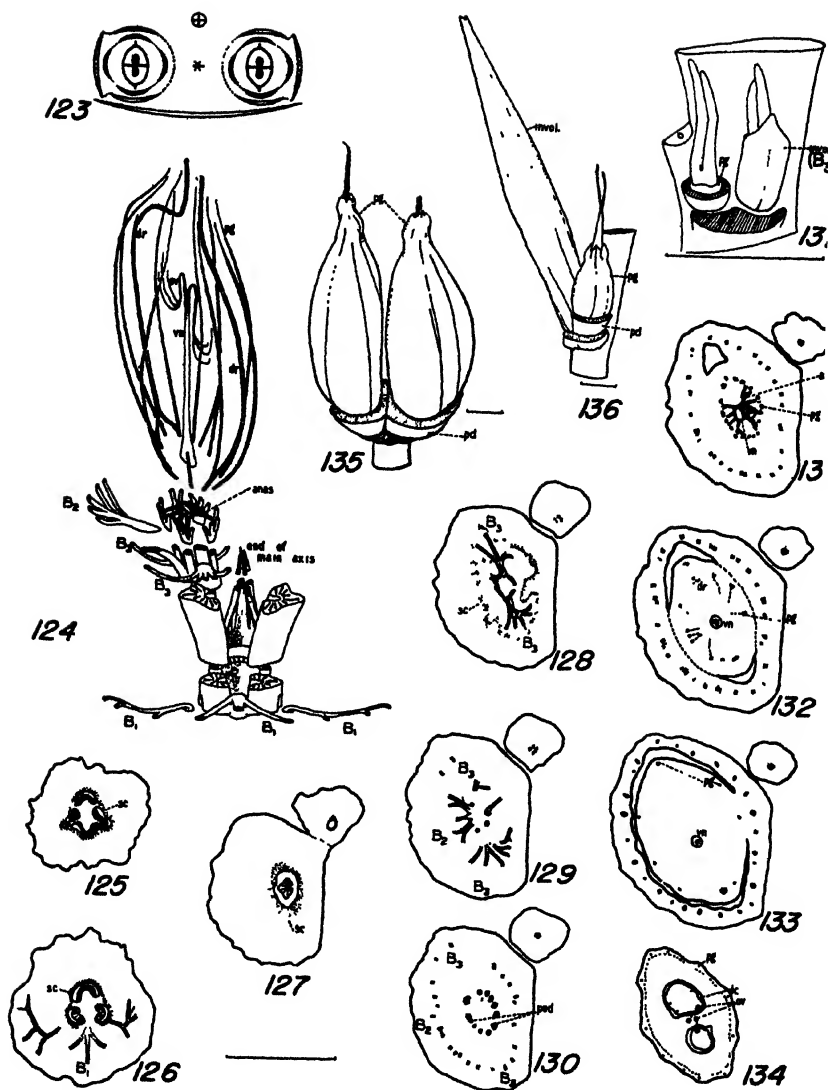
FIGS. 123–137.— Figs. 123–134, *Ostrya carpinifolia*, pistillate: fig. 123, floral diagram for *Ostrya* in general; fig. 124, reconstruction of vascular system of one-half of cymule, based on following figures; figs. 125–134, successive transverse sections through one member of a cymule (*cf.* reconstruction of vascular system in preceding figure). Figs. 135–137, *O. virginiana*, pistillate: fig. 135, cymule with involucre and primary bract completely removed; fig. 136, cymule in early stage of development with involucre cut away from one floret and primary bract removed; fig. 137, cymule at early stage of development with involucre from one floret and also primary bract removed.

Carpinus japonica has been chosen to illustrate the genus. So similar is it in the vascularization of the cymule to *Ostrya* that the reconstruction for the latter (fig. 124) will serve equally well here. The primary bract of the species studied in the s. Distego- carpus is trilacunar in its vascularization (fig. 141) while in s. Eucarpinus it is essentially unilacunar. There is a well defined secondary axis vascular system (fig. 142) which soon separates laterally (fig. 143) to make up the tertiary branches, leaving behind from their adaxial shoulders the traces *y* and *ỹ* as the sole represe- sentatives of the secondary vascular system. After persisting to the level at which the bundle of the tertiary bract departs these finally disappear (fig. 145), a type of behavior also found in *Ostryopsis*. The organization of the tertiary vascular cylinders is soon followed by the differentiation of the secondary and abaxial tertiary bract bundles (fig. 143) which depart and immediately branch (fig. 144). There is left a poorly defined tertiary vascular axis from which is derived the bundle to the free adaxial tertiary bract (fig. 146). The apparent tardiness with which this occurs is occasioned by the tilt of the tertiary axis so that the sections are not truly transverse. It is also significant that the bundle to the free tertiary bract does not branch until it is well away from the tertiary vascular system. On the other hand, that of the abaxial tertiary bract branches as it departs from the vascular cylinder of the tertiary axis which con- tributes to the vascular system of the compound bract (composed of the primary and the secondary bract). This type of behavior indicates the influence of the "fusion" of organs on the behavior of their vascular systems.

Ostrya, pistillate cymules   ·

The similarity to *Carpinus* of the species studied[7] in this genus is at once apparent. The major differences in external morphology are the shorter tertiary axes (*pd*, figs. 135, 136) and the lateral fusion of the tertiary bracts to each other as well as to the secondary bract to form an involucre (*invol*, fig. 137). In early developmental stages (fig. 136) in this genus the tertiary bracts are much more evident than is the secondary, while the reverse is the case in *Carpinus*

[7] *O. carpinifolia, O. japonica, O. knowltonii, O. virginiana.*
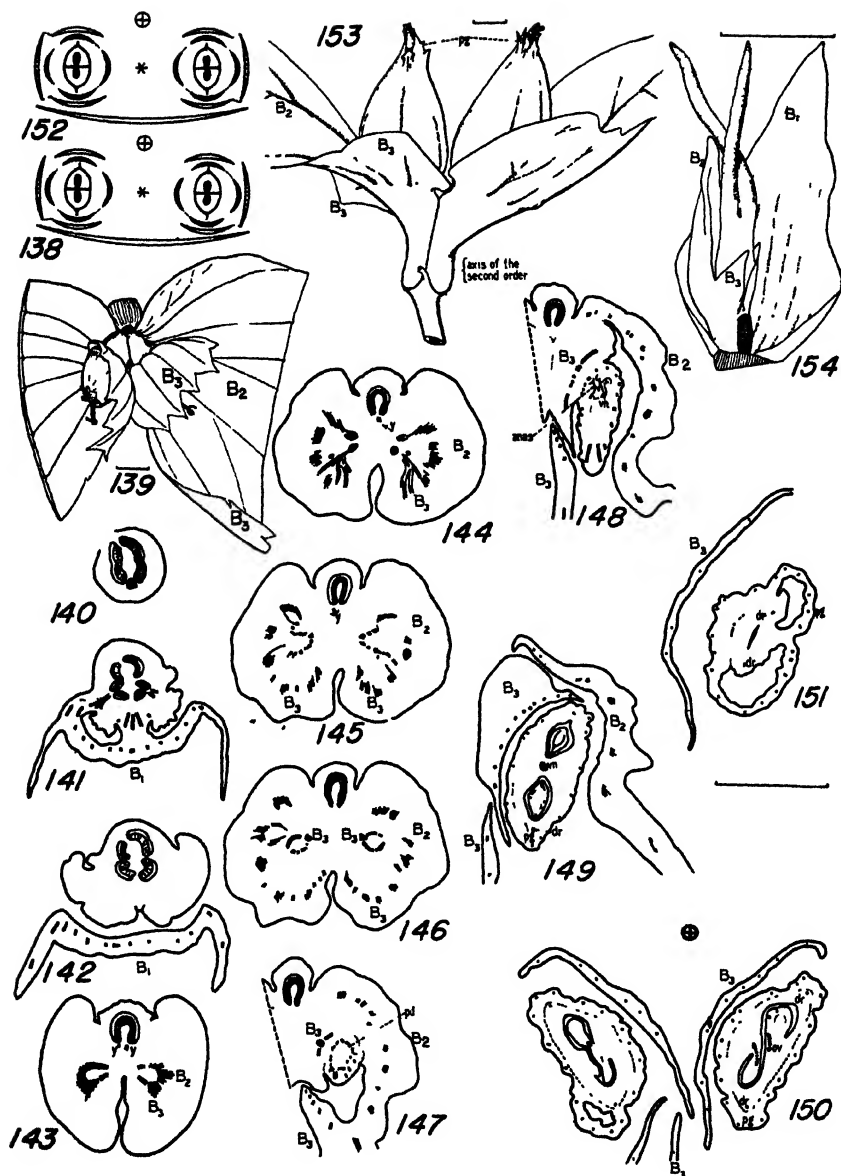
FIGS. 138–154.—Figs 138-151, *Carpinus japonica*, pistillate. fig. 138, floral diagram for following figure; fig. 139, view of cymule from above, outer portions of primary bracts removed; figs. 140–151, successive transverse sections through bract-complex shown in preceding figure (in fig. 150 both ovaries are shown). Figs. 152–154, *C. caroliniana*, pistillate: fig. 152, floral diagram for following figures; fig. 153, adaxial view of cymule, outer ends of primary bracts removed; fig. 154, early developmental stage, one member of cymule removed.

(fig. 154). This discrepancy in size is reflected in the vascular system, and in the nearly mature involucre (fig. 136) in which the two tertiary bracts are indicated by the teeth in the margin of the involucre.

The vascular system of *O. carpinifolia* is shown in a three-dimensional reconstruction in figure 124, and has been ·chosen for description because it shows most clearly the basic relationships of the various organs and their vascularization. The vascular supply to the primary bract is trilacunar (figs. 125, 126), like that of *Carpinus japonica*, while the other species are more nearly comparable to *Ostryopsis* where there is only a suggestion of a multilacunar condition. Aside from this the species are very similar in the vascularization of their cymules, except that in *O. carpinifolia* the vascular supply to the secondary and tertiary bracts is more clearly defined. In the latter the branching of the vascular system occurs so close to the vascular axis that it is often difficult to determine the individuality of the bracts. Indeed the vascular system of the secondary bract shows a strong tendency in the other species to unite with that of the adjacent tertiary bracts, suggesting the functional abortion of the secondary bract and indicating a parallelism to the situation to be considered later in *Corylus*. The length of the secondary and tertiary axes is sufficient for these to show definitely organized vascular systems. Thus there is a secondary vascular system present, at the level shown in figure 126, composed of two arcs of well developed vascular tissue. The two arcs swing laterally, leaving no vestigial tissue representative of the secondary axis, and each becomes the vascular tissue of a tertiary axis (fig. 127 shows one of the two tertiary axes in section). From the tertiary vascular system the bundles to the tertiary bracts soon depart (fig. 129). Owing to concrescence, the vascular supply to the secondary bract is tardy in its departure (fig. 129) from its prolonged association with the tertiary vascular axis. The bundles to the secondary and tertiary bracts branch almost immediately to form the many small bundles of the main portion of the involucre (fig. 129 *et seq.*). The vascular tissue remaining after the departure of the secondary and tertiary bracts is in the form of a loose dictyostele from which the floral supply is later derived.

The essentially simple nature of the vascularization of the cymule here and in *Carpinus* accords well with the hypothesis established somewhat earlier in this paper, namely, that the cymule of the Betulaceae possesses a vascular system which is fundamentally characterized by the simple relationships of bract to axillary branch slightly modified by concrescence and shortening of the internodes.

Ostryopsis, pistillate cymules

O. davidiana and *O. nobilis* in this genus were studied and found to be practically identical in their morphology and vascularization. In both species the cymules possess only the tertiary florets. The cymules are well separated from each other on the primary axis so that there are absent the dorsiventral and lateral pressures which so modify the form of the cymules in *Alnus* and *Betula*. There are but few cymules in the catkin as well. The primary bract is free from the other bracts. Lateral fusion of the secondary to the tertiary bracts (which are of about the same length) has resulted in the formation of an involucre (fig. 99). *Ostryopsis* differs from *Carpinus* and *Ostrya* further in the shortness of the tertiary axes of the cymules, resulting in a practically sessile condition of the tertiary florets.

Like the vascular supply to the cymules of the other genera described, there is formed first in the vascular cylinder of the primary axis of the ament a gap (fig. 101) with a large bundle at the base which proceeds directly into the primary bract. From the sides of the gap arise a series of traces (fig. 101) which form a dictyostelic, laterally compressed cylinder in the cortex of the primary axis (fig. 102), and from which there departs the broad bundle to the secondary bract (fig. 104) in a plane at right angles to that of the primary bract bundle. The numerous small bundles flanking this region of departure reorganize to form the tertiary vascular cylinder (fig. 105) from which very soon there depart the bundles to the tertiary bracts in a plane parallel with that of the primary bract and at right angles to that of the secondary bract. Bundles x and z (figs. 103–105) representing the secondary vascular axis ultimately disappear without taking part in the supply to any of the organs present in the cymule. Several accessory traces pass to the primary bract. This may represent amplification resulting from the working

back of the primary bract traces into the vascular system of the secondary axis, a process possibly associated with concrescence of the primary with the secondary axis.

Corylus, pistillate cymules

As in *Ostryopsis*, the species studied[8] in this genus have but few cymules in an ament, but, unlike *Ostryopsis*, these cymules are closely crowded, and only a small proportion of them mature fruit. Often in the uncultivated species and varieties it is only one of the tertiary florets which develops in a given cymule while the other floret has been arrested early (fig. 109). The successful floret then, because of its vigorous growth, deflects the smaller floret and in many cases appears to terminate the primary axis of the ament. The species studied provide a series of steps from complete freedom of the bracts of the third order to their lateral fusion and modification to the form of a campanulate tube. *C. heterophylla* (fig. 122) provides an example of the former; *C. maxima* (fig. 119), an example of an intermediate state; and *C. cornuta* (fig. 109), an example of the formation of a tube after the lateral fusion of the tertiary bracts. The secondary bracts are lacking.

The vascular systems of the different types of cymules in this genus do not differ appreciably, therefore a description of that of *C. cornuta* (fig. 118) will perhaps suffice.

Study of earlier developmental stages indicates many similarities to that of *Ostryopsis*, especially since the secondary and tertiary axes are so strikingly abbreviated. In the earlier stages of development the vascular supply to a given cymule departs from the vascular cylinder of the primary axis in the usual way, and the supply of the primary bract is independent of the rest of the vascular system of the cymule (fig. 110). The vascular supply to the rest of the cymule arises from the sides of the gap left by the primary bract bundle.[9]

These bundles form a short dictyostele representative of the

[8] *C. americana, C. avellana, C. californica, C. cornuta, C. colurna, C. davidiana, C. heterophylla* var. *sutchuensis, C. tibetica, C. vilmorinii, C. maxima.*

[9] This condition is obscured in the series shown in figs. 110-117 because of the great development of one of the florets at the expense of all the other structures in the ament, so that the proportions are tremendously modified.

secondary axis, which broadens laterally and separates into two arcs, one for each tertiary axis. But at this time there is no evidence of a vestigial vascular supply to the lost secondary bracts. Between the two arcs there remain the traces y, y and z, z which are the sole remnants of the secondary vascular axis. These traces ultimately migrate back toward the primary vascular axis and there take up a position in the gap left by the departure of the vascular tissue to the cymule. The two arcs of tissue representing the two tertiary axes tend to form dictyosteles, and at the same time a broad band of vascular tissue passes off to the abaxial tertiary bract. At a slightly higher level the traces to the adaxial tertiary bract pass off leaving behind a very short pedicellar cylinder of vascular tissue.

At the developmental stage shown in the reconstruction in figure 118 and in figures 110–177, the vascular supply to the other floret of the cymule is shown departing to the left in figure 110, while the vascular supply to the undeveloped distal portion of the ament is shown toward the top of the figure. The cylinder of tissue in figure 111 is the representative of the tertiary axis of the larger floret only in the cymule shown in figure 109. This dictyostele rapidly breaks up (fig. 112), sending off branches centrifugally to supply the tertiary bracts and also branches centripetally which represent the continuation of the tertiary vascular axis into the base of the floret. The system is much modified by anastomoses (*anas*) which enter into the ovary supply and will be considered in a discussion of the vascularization of the florets of the Betulaceae. With the increase in size of the cymule the bundles of the tertiary bract branch more profusely. A similar tendency has been noted in *Carpinus*, *Ostrya*, and *Ostryopsis*, where there is great development in size of the floret ovary in close proximity to the place of departure of the bract bundles from the very short axes of the cymule.

The dissection of the vascular system of the pistillate cymule in *Corylus* developmental stages appears to be associated with the extraordinary increase in the size of one of the two florets of the cymule in a notably telescoped inflorescence. Since the full influence of such factors as size on the internal morphology of the vascular system is by no means clear, although its significance is recognized,

there is no accepted basis for attributing definite cause-effect relationships to such coincidences as have been observed.

In general, however, the vascular system is essentially that of a cymule in which, surprisingly enough, the secondary bracts are absent, and in which there is excessive development in size of one of the florets of the cymule, resulting in disruption of the symmetry of the vascular system. The greatest similarity is with the vascular supply of *Ostryopsis* since there is in both the same abbreviation of the secondary and tertiary axes. This may be a simple case of parallelism, however, since the striking difference in the presence or absence of the secondary bract must be considered as well.

STAMINATE CYMULES: MORPHOLOGY AND ANATOMY

While there is much variation in the gross morphology of the pistillate ament, there is marked uniformity in that of the staminate ament, and it is here that the popular concept of the ament finds justification. The staminate aments are elongate, pendulous, and in many cases deceivingly simple in the morphology of their component cymules. In these cymules much variation from one group of species to the next is to be found, although all three florets are almost universally represented. This will be treated in some detail under the various genera, but basically the staminate cymules adhere to the interpretation accorded the pistillate cymules and the same principles of interpretation of the vascular system may be used in both cases.

From the anatomical point of view, the species studied possess in common the absence of secondary thickening in the xylem, both in the primary axis and in the axes of the higher orders. The evidence indicates that the vascular relationships of the leaf to the axillary branch in a system where concrescence plays a prominent rôle is applicable throughout. Absence of secondary thickening appears to be correlated with the time factor, since secondary xylem is also lacking at a corresponding period in the development of the pistillate aments. Owing to the fall of the staminate aments after the shedding of the pollen there is of course no further opportunity for secondary xylem to form.

Alnus, staminate cymules

A study of a number of species[10] indicates that there is a great range of variation in the morphology and anatomy of the individual cymules of this genus.

The habit sketches of a cymule of *A. firma* var. *hirtella* (figs. 156, 157) are representative of the members of s. Gymnothyrsus. In addition to the primary bract, both secondary bracts are present but only one of the tertiary bracts occurs. Other cases have been found in which both of the abaxial tertiary bracts (the adaxial bracts are uniformly absent) are present, or more rarely both abaxial tertiary bracts may be absent.

The vascularization of a cymule of *A. crispa* var. *mollis* is shown in figure 159 and the following transverse sections (figs. 160–167). The vascular supply to a cymule originates much as it does in the pistillate material, a single trace deriving from the base of an elongate gap in the vascular cylinder of the primary axis. This trace grows larger as it passes outward into the base of the cymule, becomes cylindrical by the overarching of its margins (fig. 160), and then separates into (1) an upper portion which represents the continuation of the secondary axis and which ultimately supplies the secondary floret (y and y, fig. 161), and (2) a lower portion which continues for a time in a loose association with the other vascular tissue (figs 161, 162) but soon becomes independent (fig. 163) and ultimately supplies the primary bract (fig. 164 *et seq.*). From the vascular tissue flanking the primary bract bundle there differentiates the secondary bract bundle, which has associated with it the various traces which supply the tertiary florets and represent the much telescoped tertiary vascular axis. Where the tertiary abaxial bracts are present they derive their vascular supply from between the secondary and primary bundles, just as in the pistillate cymules.

[10] Section Alnobetula: *A. firma* var. *hirtella, A. crispa* var. *mollis, A. sinuata.*
　Section Gymnothyrsus: *A. incana, A. jorullensis, A. hirsuta, A. hirsuta* var. *sibirica, A. tenuifolia, A. spaethii, A. rhombifolia, A. rubra, A. maritima.*
　Section Clethropsis: *A. nitida, A. nepalensis.*
　Section Cremastogyne: *A. cremastogyne, A. lanata.*
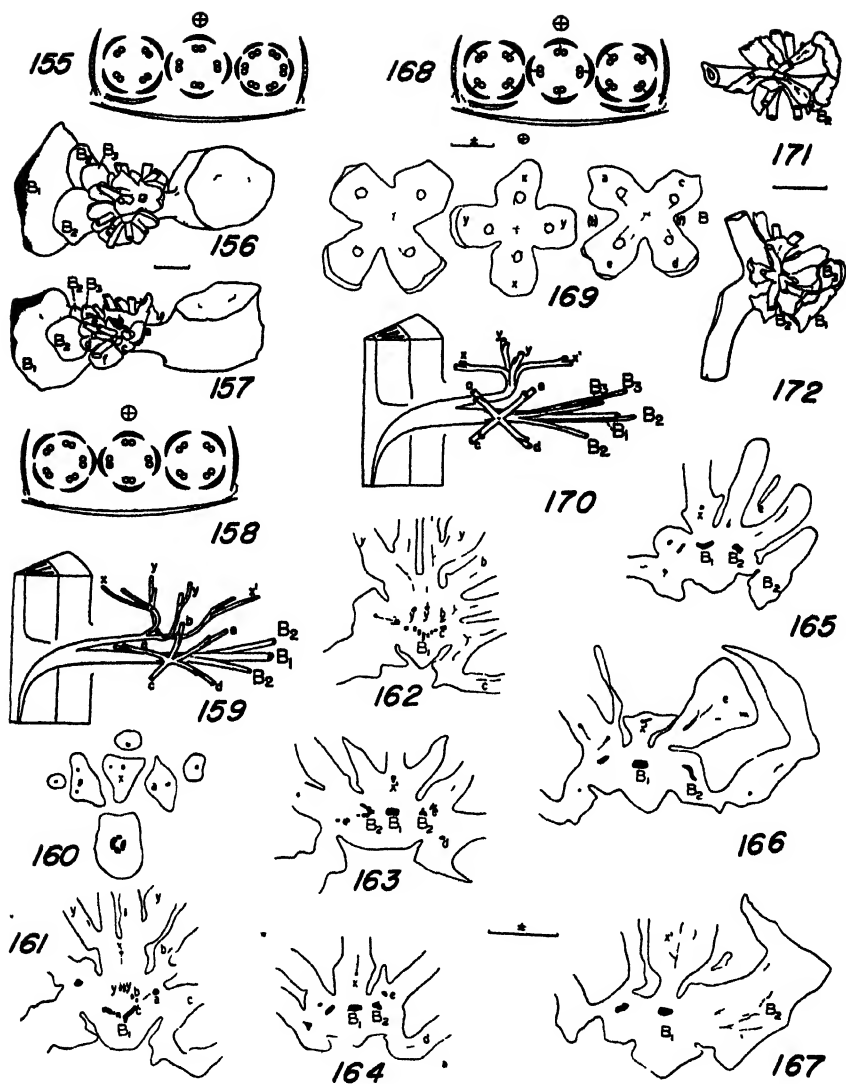
FIGS 155–172.—Figs 155–157, *Alnus firma* var. *hirtella*, staminate: fig. 155, floral diagram for two figures following; fig. 156, dorsal view of cymule*; fig. 157, lateral view. Figs. 158–167, *A. crispa* var *mollis*, staminate: fig. 158, floral diagram of cymule shown in following figures; fig. 159, reconstruction of vascular system of cymule shown in following figures; fig. 160–167, successive transverse sections. Figs. 168–170, *A. incana*, staminate. fig. 168, floral diagram of cymule shown in following figure; fig. 169, the three florets removed and placed in one plane in normal orientation to one another; fig. 170, reconstruction of vascular system. Figs. 171, 172, *A. nitida*, staminate: dorsal and lateral views respectively.

* The anthers have been removed in practically all staminate cymules figured so that filaments only represent the stamens

A. firma var. *hirtella* departs in certain respects from the preceding type of vascularization in that the origin of the vascular supply to the cymule is three-parted, as it is in the typical branch-bract association, the median bundle ultimately supplying the primary bract and the two bundles arising from the sides of the gap providing the vascular supply to the rest of the cymule. The bundles z and z, mentioned in connection with the anatomy of the pistillate cymules of *A. subcordata, Corylus* and *Ostryopsis*, appear in this species also and replace in their function the bundles y and y. The presence of z and z on either side of the primary bract bundle supplies a portion of the secondary axis vascular system lost in the other species of this section. Furthermore the trace e in this species arises from between the bundles to the secondary and tertiary bracts, which emphasizes the similarity between the staminate and pistillate vascular systems.

As shown in the reconstruction (fig. 170), the vascular system of a cymule of *A. incana*, representative of most of the species of s. Gymnothyrsus, is essentially like that of the species of the preceding section except that the tertiary bracts are regularly present. The secondary floret, as usual, is supplied by the traces x and y, y representing the secondary axis. The tertiary axis is composed of the bundles a and c arising from the ventral flank of the secondary bract trace, and by c and d arising from between the secondary bract trace and the tertiary bract trace. In *A. rhombifolia* and *A. jorullensis* the secondary axis is reduced to even greater simplicity by the presence of only the trace x, while the tertiary axis is represented only by the fusion bundle e plus d which arises from between the bundles supplying the secondary and tertiary bracts of the floret involved. *A. rubra* differs from the rest of the *A. incana* group in the formation of the secondary axis from the traces x and y, the last arising from the sides of the primary bract trace.

In only one case has a cymule been found in which the secondary floret was lacking, thus presenting a similarity to the regular pistillate condition. In the distal portion of the ament in *A. tenuifolia* one such cymule was found, which upon sectioning showed a condition like that of the three-flowered cymules. Here the traces x, y, and y after becoming differentiated become closely approxi-

mated with the bundles to the tertiary bracts, much as do the homologous traces in the normal two-flowered pistillate material.

The cymules of the species composing s. Clethropsis are strikingly different from those of the preceding two sections in the marked shortness of the secondary and tertiary axes, bringing the florets very close to the primary axis of the ament (figs. 171, 172). In both species, bracts of the second and third order are present in addition to that of the first order, but the range of floret conditions covers that of the species of both the preceding sections. In the distal portion of the aments of *A. nepalensis*, the bracts of the third order and then those of the second order are absent from the cymules. There are corresponding reductions in the number of parts present in the individual florets.

The vascular system of the cymules of the species studied from this section is essentially like that of the preceding two sections, except that, as in the pistillate cymules of these species, there is early division of the bundles ultimately supplying the cymule, so that there is a well defined dictyostele in the short secondary axis.

Both species of s. Cremastogyne agree well in the morphology of their cymules. This is illustrated for *A. lanata* in figures 177 and 178. There is no external evidence of a condition similar to that just described for the cymules of the terminal portion of the ament of *A. nepalensis*. The vascular system is shown in figure 179. The supply to the cymule originates as a single small mass of xylem at the base of a gap.

A dictyostele forms by the overarching of this small bundle, while the traces y and y, and x depart in the usual way to form the vascular system of the secondary axis. The bundle supplying the primary bract differentiates opposite to x and supplies the primary bract. This bundle is flanked by two diminutive bundles which parallel it and doubtless represent the vestiges of the supply to lost secondary bracts since the trace d departs from the outer side of it. Trace d represents the sole remaining portion of the tertiary vascular axis.
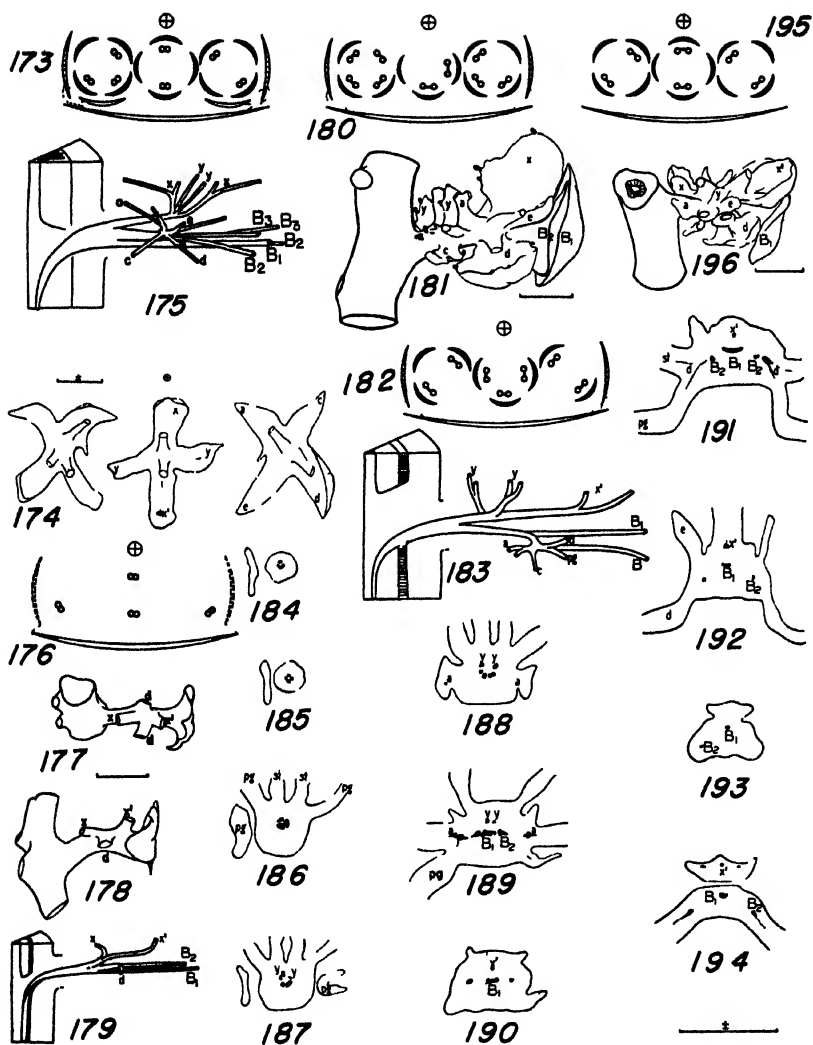
FIGS. 173-196 —Figs. 173-175, *Alnus rhombifolia*, staminate fig 173, floral diagram for following habit sketch; fig. 174, three florets of cymule dissected off and shown in relative positions; fig. 175, reconstruction of vascular system of cymule similar to that shown in preceding figure. Figs. 176-179, *A. lanata*, staminate· fig. 176, floral diagram of cymule; figs. 177, 178, dorsal and lateral views respectively; fig. 179, reconstruction of vascular system of cymule shown in preceding two figures. Figs. 180, 181, *Betula lenta*, staminate: floral diagram and lateral view of cymule respectively. Figs. 182-194, *B. lutea*, staminate: fig. 182, floral diagram; fig. 183, reconstruction of vascular system of cymule (very similar to that of *B. lenta*); figs. 184-194, successive transverse sections upon which preceding reconstruction is based. Figs. 195, 196, *B alnoides* var. *pyrifolia*, staminate· floral diagram and lateral view respectively.

Betula, staminate cymules

A general survey of the cymules of the species studied[11] in this genus shows the striking similarity between the cymules of the birches and those of some of the alders. The condition which is typical in *Betula* has been foreshadowed and in some cases surpassed in *Alnus*, namely, the absence of the tertiary bracts. As in the s. Cremastogyne of *Alnus*, even the secondary bracts are absent in the ss. Acuminatae of *Betula*.

A consideration of the anatomy of the cymules of the genus may well be based on that of *B. lutea* (fig. 183). The vascular system to a cymule originates as a single strand from the base of the gap. This strand becomes augmented as it approaches the cortex, there becomes lunate, and finally forms a loose siphonostele out in the basal portion of the cymule (fig. 184). This small vascular cylinder is that of the secondary axis of the cymule. From the upper portion of this loosely organized cylinder there depart the two traces y and y representing the continuation of the secondary vascular system beyond the differentiation of the tertiary vascular axis (figs. 186–188). It is from these traces that the supply to the secondary floret is derived. The remaining vascular tissue of the cymule consists of the primary bract bundle, which because of concrescence is thus long associated with the vascular system of the cymule, and two more or less well defined arcs of tissue flanking the secondary bract bundle on either side. These arcs represent the two tertiary vascular axes from which the vascular tissue of the tertiary florets and secondary bracts is derived in much the same way as in *Alnus*. The major

[11] Section Eubetula:

Subsection Costatae:	*B. lenta, B. lutea, B. grossa, B. nigra.*
Subsection Albae:	*B. coerulea-grandis, B. japonica, B. papyrifera* var. *carpatica, B. papyrifera* var. *occidentalis, B. pendula, B. populifolia.*
Subsection Nanae:	*B. glandulosa, B. michauxii, B. pumila.*

Section Betulaster:

Subsection Acuminatae:	*B. alnoides* var. *pyrifolia, B. luminifera, B. maximowicziana* (material of this species is from a tree in the Arnold Arboretum and is almost surely hybrid, the female parent being *B. maximowicziana,* the male parent unknown).

FIGS. 197–221.—Figs. 197–208, *Betula pendula*, staminate: fig. 197, floral diagram; fig. 198, dorsal view of cymule; fig. 199, lateral view of reconstruction of vascular system; figs. 200–208, successive transverse sections through cymule similar to that shown in fig. 198. Figs. 209–218, *B. pumila*, staminate: fig. 209, floral diagram; fig 210, dorsal view of cymule; fig. 211, reconstruction of vascular system similar to that shown in preceding figure; figs. 212–218, successive transverse sections. Figs. 219–221, *B. maximowicsiana*, staminate cymule: dorsal and lateral views and floral diagram respectively.

difference is that the tertiary vascular system in *B. lutea* is represented by only a single compound bundle derived from the upper shoulder of the secondary bract bundle.

There are few departures from this general plan. Only in *B. maximowicziana* is the bundle *x* found to be present as it occurs in the staminate cymules of *Alnus*. In the species of ss. Acuminatae which have lost the secondary bracts there is not even a vestigial secondary bract trace, but otherwise the vascular system resembles that described for *B. lutea*.

Thus it is clear that a notable uniformity of vascular plan characterizes the genus as a whole, with the species of ss. Acuminatae standing apart because of the absence of the secondary bracts and the corresponding bract bundles, a condition parallel to that occurring in the s. Cremastogyne in *Alnus*.

Carpinus, staminate cymules

Of the species studied,[14] the most significant in view of what has gone before is *C. japonica*, since there one finds in the cymules at the base of the aments the free secondary bracts (fig. 239). That these are indeed bracts and not stipules is shown by their presence in addition to stipules (fig. 241) in the cymules from a few nodes farther down in the transitional region between twig and ament. At higher levels (figs. 237, 235) these secondary bracts are no longer distinguishable externally. In the other species of the genus the cymules are similar to the examples illustrated (figs. 232, 234). Confusion in the interpretation of the morphology of the cymule in this and the following genera has long existed because of the absence of tepals in the normal florets. It is clear from the vascular systems, however, that there are actually three florets composing each of the cymules. As indicated in the habit sketches, the bracts are richly supplied with small veins. While these are referable to three main systems corresponding to the primary and the two secondary bracts, this is obscured by their branching at a very early stage, recognizable in most cases only by sectioning.

[14] Section Eucarpinus: *C. betulus, C. caroliniana, C. laxiflora, C. orientalis, C. turczaninowi.*
Section Distegocarpus: *C. japonica, C. cordata.*
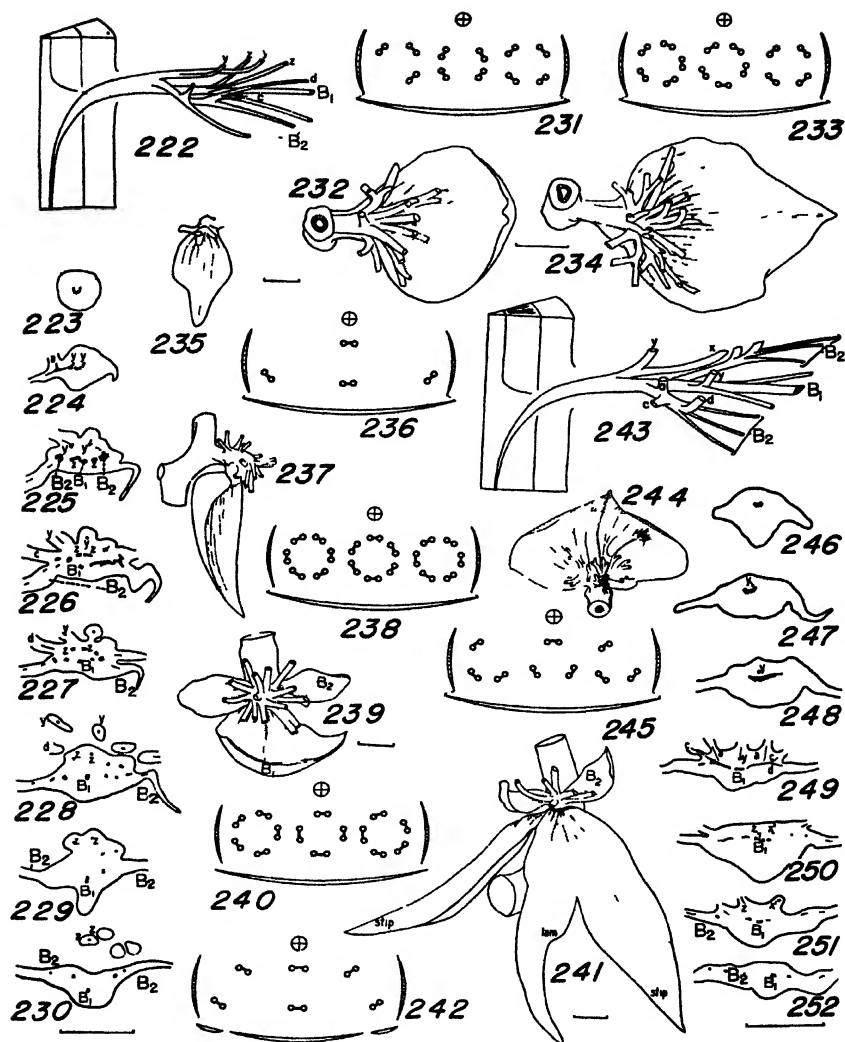
FIGS 222–252 —Figs 222–230, *Carpinus betulus*, staminate; fig 222, reconstruction of portion toward the observer of vascular system, based on figs. 223–235; figs. 223–230, successive transverse sections through cymule similar to that shown for *C laxiflora* Figs 231, 232, *C. laxiflora*, staminate. floral diagram and dorsal view of cymule respectively. Figs 233, 234, *C. caroliniana*, staminate cymule: floral diagram and dorsal view respectively. Figs 235–242, *C. japonica*, staminate cymules: figs 235, 236, dorsal view and floral diagram respectively from terminal portion of ament; figs 237, 238, lateral view and floral diagram from central portion of ament; figs 239, 240, dorsal view and floral diagram respectively from near base of ament (note free secondary bracts); figs. 241, 242, dorsal view and floral diagram respectively in transition region between ament and twig (note reduced lamina, *lam;* persistent stipules, *stip;* and free secondary bracts) Figs. 243–252, *Ostrya virginiana*, staminate cymule: fig 243, reconstruction of vascular system shown in section in following figures; figs 244, 245, dorsal view and floral diagram; figs. 246–252, successive transverse sections similar to that shown in fig 244.

The vascular system of *C. betulus* (fig. 222) characterizes that of the other species of the genus. The origin of the vascular system, like that of *Betula*, is in a single small bundle which arises at the base of a gap. This trace becomes lunate (fig. 223) but not siphonostelic, and from the upper margins gives off the two bundles *y* and *y* (fig. 224) of the secondary axis. The trace to the primary bract arises from the median lower portion of the vascular system (fig. 225), and on either side there arise the traces *z* and *z*, which constitute the remainder of the vascular system belonging to the secondary axis (figs. 225–228). Laterally there departs the vascular tissue composing the tertiary axis and that of the secondary bract associated with it (fig. 226 *et seq.*). So similar is this behavior of the vascular system to that obtaining where there are obviously three florets present that there can be no doubt that here there are also three florets bearing the same relationship to the cymule as do the florets in the other genera. This is emphasized by a parallel case observed in the staminate cymules of *Alnus*, s. Cremastogyne, and in the pistillate cymules of *Betula*, ss. Clethropsis. Furthermore the vascular system of the cymules with free secondary bracts in *C. japonica* (figs. 239, 241) differs in vascularization from the cymules of the other species in that the secondary bract traces pass to organs which are free rather than fused laterally to the primary bract. That tertiary florets occur in the axils of these secondary bracts is clear because of the close relationship between their respective vascular systems.

Ostrya, staminate cymules

The species studied are those noted under the description of the pistillate cymules (see footnote 7).

The external morphology, together with the distribution of vascular tissue of the cymules in this genus, is so like that in the majority of species described for *Carpinus* that no further description is necessary. A reconstruction of the vascular system and serial transverse sections are provided in figures 243, 246–252.

Corylus, staminate cymules

The species studied are those mentioned in the description of the pistillate cymules (see footnote 8). This genus presents an interesting and unique morphology. The secondary bracts, which are

regularly present, are well developed and dorsiventrally fused to the primary bract. The individual florets are difficult to distinguish because they lack tepals and because the secondary floret is dimerous while those of tertiary origin are monomerous. The vascularization of the cymules, however, definitely establishes the fact that there are three florets involved. The vascular system originates as a single bundle from the base of the gap in the primary vascular cylinder, proceeds out into the base of the cymule, and there forms a diminutive siphonostele (figs. 260, 261). From the upper portion of this stele there departs the single bundle x, which is all that remains of the pedicellar portion of the secondary vascular axis (figs. 261, 262), while the remainder of the vascular tissue flattens out dorsiventrally (figs. 262, 263). From each side of this flattened vascular mass a secondary bract bundle separates. At the same time the trace c, representative of the tertiary axis, departs laterally from the secondary bract bundle (figs. 264, 265). From the space between the secondary and primary bract bundles originates the trace z, homologous with the same trace in the pistillate material of this genus, which completes the secondary vascular system. Thereafter the primary and secondary bract traces proceed unmodified except for branching into their respective bracts (fig. 265 *et seq.*).

The presence of secondary bracts and the intimate relation between their vascular supply and that of some of the floral members indicate that these members are to be considered the tertiary florets, which strengthens the conclusion already reached that the staminate cymule of *Corylus* is three-flowered. Furthermore a striking similarity exists between the vascular system of the cymules here and those in the preceding two genera, so that the evidence concerning the interpretation of the cymule as three-flowered is mutually corroborative.

Ostryopsis, staminate cymules

The same species were studied as in the pistillate material.

Morphologically (figs. 268, 270) and anatomically (fig. 272) the cymules are very simple in this genus. Essentially the same condition exists as in the cymules from the terminal portion of the aments in *Carpinus japonica* previously described. No secondary bracts nor traces to secondary bracts are present, and the members
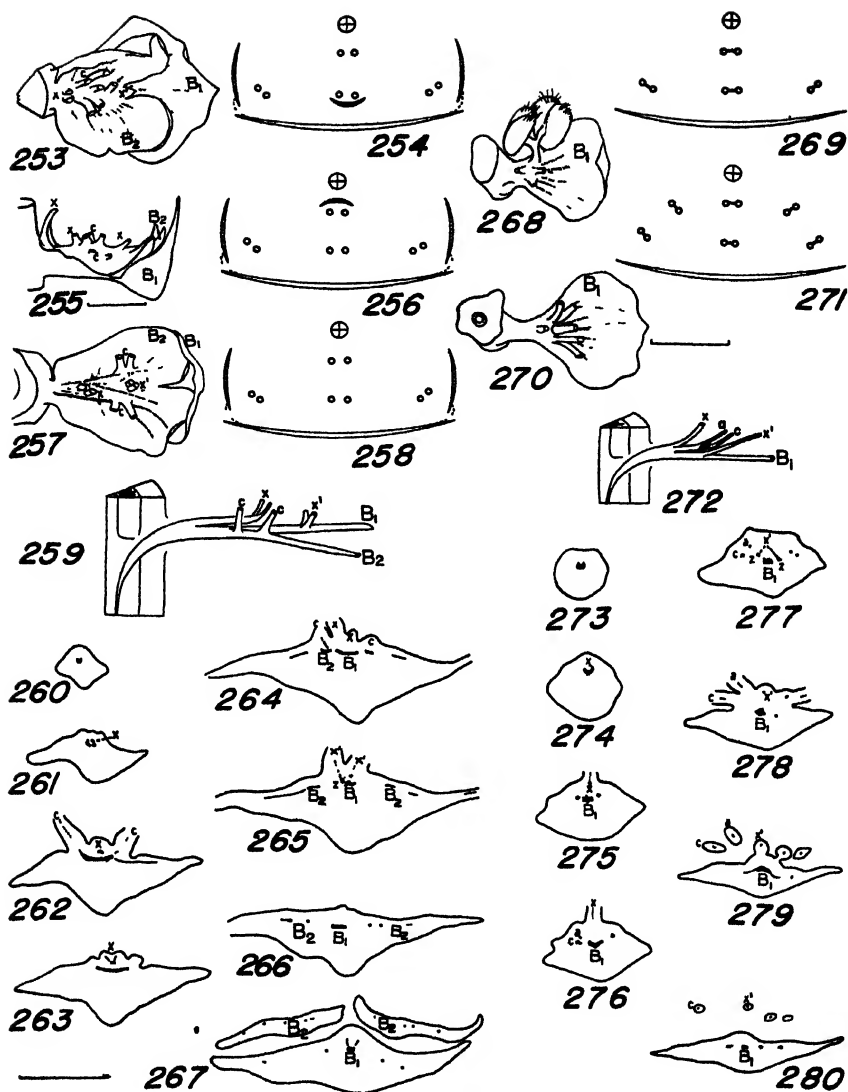
FIGS. 253–280.—Figs. 253, 254, *Corylus americana*, staminate cymule from near proximal portion of ament, dorsal view and floral diagram respectively. Figs. 255, 256, *C. maxima*, staminate cymule from proximal portion of ament, lateral view and floral diagram respectively. Figs. 257, 258, *C. heterophylla* var. *sutchuensis*, staminate cymule showing usual condition in the genus, including preceding two species. Figs. 259–267, *C. cornuta*, staminate cymule: fig. 259, reconstruction of vascular system; figs. 260–267, successive transverse sections. Figs. 268–280, *Ostryopsis davidiana*, staminate cymules: figs. 268, 269, dorsal view and floral diagram respectively with but a single stamen in each of tertiary florets; figs. 270, 271, dorsal view and floral diagram with two stamens in each of tertiary florets; fig. 272, reconstruction of vascular system of type shown in fig. 270; figs. 273–280, successive transverse sections.

of the central floret are supplied by the traces x (figs. 274, 275) and
z (fig. 277) while those to the tertiary florets are supplied by the
traces a and c (fig. 276). The course and origin of the vascular sys-
tem in the cymules of this genus are simplified versions of those
described for the preceding three genera. This genus apparently
reaches the ultimate in simplification of the staminate cymule by
reduction for this family.

<center>SUMMARY OF CYMULE MORPHOLOGY AND ANATOMY</center>

In the interpretation of the inflorescence anatomy of the Betu-
laceae it is necessary to postulate only that the inflorescence is
essentially a branch system. In utilizing this hypothesis the simple
facts of nodal anatomy as set forth in the introduction constitute
the working premises. In essence these are as follows: (1) that in a
unilacunar condition the vascular supply to the bract arises from
the base of the gap, while the vascular system of the axillary branch
is derived from the mutual overarching of two bundles, derived from
opposite sides of the gap (fig. 1); (2) that under conditions in which
the internodes are short and the gaps do not close, the vascular
cylinders become dictyostelic (fig. 2); (3) that if concrescence of the
bracts is superimposed upon this, the bract traces appear to depart
from the vascular cylinder of the axis of the next higher order
(fig. 3); (4) that when dorsiventral and lateral pressures are in-
troduced the courses of the bundles to the bracts are modified as the
positions of the bracts are modified although their origins are but
little affected (fig. 4); and (5) that it would seem a logical corollary
of (4) that where pressure is greatest the organs are most likely to
be suppressed and their vascular system ultimately lost as well.

In the Betulaceae, in the pistillate cymules of *Alnus* when these
cymules are three-flowered (fig. 17)—as in proliferated material- -
the nearest approach (fig. 19) is made to a simple type of vascular
system, such as that shown in figure 3. In the absence of the second-
ary floret (fig. 21) the traces x (fig. 20) and y (fig. 65) still exist as
vestigial traces representing the secondary vascular system; and as
is often the case with vestigial vascular bundles, they tend to fuse
with certain of the adjacent vascular bundles. The reverse of this
condition is found in the staminate cymules of *Alnus*, which are

regularly three-flowered (fig. 168) and which have the complement
of vascular tissue (fig. 175) associated with the pistillate three-
flowered cymules. In the one case found in which the secondary
floret of the staminate cymule was absent, however, the vestigial
bundles of the secondary vascular axis behaved very like that of the
two-flowered pistillate cymules.

Associated with the tendency toward reduction, the vascular
system shows corresponding modifications, namely, that with the
disappearance of a bract its bundle also tends soon to disappear
(phylogenetically speaking). Thus the loss of the secondary bracts
(fig. 176) in the staminate cymule of the species of s. Clethropsis is
correlated with a strong reduction in the size of the bundle to this
organ (fig. 179).

In *Betula*, the same general plan (figs. 197, 77) obtains in both
the staminate and pistillate cymules, with the tertiary bracts lost
completely and the secondary bracts in the process of disappearance
in some of the species (fig. 88). Associated with this is a decided re-
duction in the entire extent of the vascular system (figs. 67, 183), as
well as in the general habit of growth of the aments which are not so
long-lived as they are in *Alnus*. There is a strong similarity between
the two genera in their external morphology and in the distribution
of their vascular tissues, indicating a common origin.

In *Carpinus* many features are found which suggest a close rela-
tionship to some less modified ancestral type, especially in the free-
dom of the several bracts from one another in the pistillate cymules
of some of the species. There are other stages (figs. 138, 152) show-
ing tendencies toward fusion of the tertiary bracts laterally to those
of secondary nature, but in their vascular organization such cases
are closely similar to those in which the bracts are free. Correspond-
ing tendencies (figs. 238, 240) were noted in the staminate cymules,
although no evidence was found of the presence of tertiary bracts.
In at least one species, however, secondary bracts were found to be
free from the primary bract in the basal portion of the ament
(fig. 240), thus explaining the presence of the vascular supply to
the secondary bract in other forms in this genus even when there
appears to be no external evidence of such a bract. This may be
attributed to the lateral fusion of these organs with the primary

bracts, just as the tertiary bracts fuse laterally with the secondary bracts in the pistillate cymules.

The absence of the tepals, which provide the most obvious means of differentiating these closely crowded florets aside from the vascular system, has misled those who have studied only the external morphology of the staminate cymules (fig. 232) of *Carpinus*. The vascular system (fig. 222), however, is so similar to that of other members of the family (fig. 175) where there is indubitable evidence of the presence of the three florets in the cymule, that there can be little hesitation in according a similar interpretation to these.

Ostrya so closely follows the lead of *Carpinus* that it needs but little comment. The pistillate cymules have proceeded but a step beyond those of *Carpinus* in that the tertiary bracts are fused laterally to each other as well as to the secondary bracts, so that each of the two florets of the cymule is surrounded by an involucre (fig. 136). Anatomically (fig. 124) this condition is close to that of *Carpinus*. The staminate cymules also are like those of *Carpinus* morphologically (fig. 244) and anatomically (fig. 243), and admit of a similar interpretation (fig. 245).

Ostryopsis is of interest because, although the pistillate cymules (fig. 99) are intermediate between *Carpinus* and *Ostrya* in the behavior of their bracts, in that the tertiary bracts are fused laterally to the secondary bract, the bracts are all of the same length and are furled around the individual florets, thus simulating the condition found in *Ostrya*. But the secondary and tertiary inflorescence axes are so shortened that the individual florets are practically sessile on the primary axis of the ament. It is only the vascular system which indicates that the various organs bear the same relationship to each other as they do in *Carpinus* and *Ostrya*. In this reduction of the tertiary axes the genus bears a closer similarity to *Corylus* than it does to the preceding two genera. Also in the morphology (figs. 268, 270) of the staminate cymules it bears a similarity to *Corylus*, although this will become more obvious after the anatomy and morphology of the individual florets have been described. There is, moreover, no external nor internal (fig. 272) evidence in the staminate cymules of the existence of tertiary or secondary bracts, in-

dicating an extreme of reduction of these organs similar to that found only in the pistillate cymule of *Betula michauxii* (fig. 85).

Corylus stands apart as an unusual genus although it bears some likeness to the other genera already mentioned. The individual pistillate cymules (fig. 122), of which there are but few in an ament, lack the secondary florets as do the pistillate cymules of the preceding three genera. In the uncultivated species, one of these tertiary florets ordinarily gains the ascendency in the course of development. An additional peculiarity is the absence of the bracts of the second order, a condition which results in the formation of an involucre composed entirely of tertiary bracts. Thus the involucre in those species in which the bracts have "fused" laterally (fig. 109) instead of remaining free (fig. 122) is by no means homologous with that of *Ostrya* (fig. 136) which it resembles superficially. Not even the vascular system of the secondary bract is present in *Corylus* (fig. 118) as it is in the involucre of *Ostrya*. The staminate cymules on the other hand possess the secondary bracts which are fused dorsiventrally to the primary bracts but lack the tertiary bracts. In addition all three florets of the cymule are represented (fig. 258).

In all these cases it is found that the mode of origin of the vascular system to the cymules, pistillate or staminate, is remarkably uniform, in many cases being of the most diagrammatic nature. While the primary bundle trace often remains in close apposition to the rest of the vascular tissue of the cymule, its identity and distinctness is never to be mistaken and readily lends itself to interpretation as the result of concrescence of that organ to the remainder of the cymule. The vascular tissue usually forms a definite siphonostele, or at least a dictyostele representative of the secondary inflorescence axis. From this siphonostele there depart laterally the bundles to the secondary bracts. Concrescence again plays a part here, so that the secondary bract trace often maintains a close relationship with the tertiary vascular system until after the departure of the tertiary vascular system from the secondary vascular axis. So intimate is the union of the vascular tissue of the tertiary axis with that of the secondary bract, that it often appears to be derived laterally from the secondary bract trace, a condition which nevertheless maintains the basic relationship between the vascular system of a bract and

its axillary branch. When tertiary bracts are present, they depart just as might be expected in a telescoped cymule, being closely associated with the vascular tissue of the tertiary axis as illustrated in the hypothetical case in figure 4.

Thus it is evident that the vascular system of the cymule, pistillate and staminate, supports the accepted interpretations of morphology in more obvious forms, and makes clear the basic morphology in more obscure forms.

Floret morphology and anatomy

Floral morphologists have long endeavored to explain the nature of the florets in the Betulaceae. In the one group, the Coryleae, the ovary has been accepted as inferior because there is tangible evidence of the presence of a perigon. But in the Betuleae, while the question has been raised of the apparent absence of perigon in a group of genera obviously close to the Coryleae, the answer has never been attempted in more than one or two isolated cases, nor has a study been made of the vascularization of the florets.

A second problem is that of the diverse orientation of the ovaries in the secondary pistillate florets, which have ordinarily been described as median to the secondary bracts in *Alnus*, *Betula*, and *Corylus*, and transverse in *Ostrya* and *Carpinus*.

The problem of the basic morphology of the florets of the Betulaceae may best be attacked by first considering the morphology and anatomy of the staminate florets.

STAMINATE FLORETS: MORPHOLOGY AND ANATOMY

Alnus, staminate florets (for species studied see footnote 10).

The individual florets (of which three are present in each cymule) in this genus regularly have a well developed perigon, with the exception of the species of s. Cremastogyne. The number of tepals varies from six to one, depending on the species. The florets of the species of s. Alnobetula are hexamerous to tetramerous, the range being shown in the single cymule of *A. firma* var. *hirtella* in figures 156 and 157 (*cf.* floral diagram, fig. 155). The hexamerous condition is rather uncommon, but when present (fig. 157) the tepals are in two whorls of three members each, as are also the stamens. No

unusual condition exists in the vascular supply to these floral com-
ponents, other than the fusion of the stamen traces to the tepal
traces somewhat beyond the point of origin of the tepal traces. Be-
yond the departure of the stamen trace, the tepal trace becomes
tripartite (fig. 160). In s. Gymnothyrsus, the individual florets are
commonly tetramerous as in *A. incana* (fig. 169); but in some species,
such as *A. rhombifolia* (fig. 174), there is a strong tendency toward
trimery or dimery of the stamens, and also in some cases toward the
reduction of the number of perigon segments. In *A. incana* the
fusion of the stamens to the tepals is so complete that it involves
the entire filament, but otherwise the vascular system of the floret
(fig. 170) is normal. In the vascular system of a floret in which the
stamens confronting certain of the tepals may be lacking, the vascu-
lar system of such tepals is generally developed as strongly as though
the stamen associated with it were present. In the reduction of the
components of the secondary floret, it is the lateral stamens which
are first lost.

The individual florets of s. Clethropsis show many similarities
to those of the two sections just mentioned, in that the whole range
from pentamery to dimery is covered. They differ in the smaller
absolute magnitude of the mature florets, and in the greatly ex-
aggerated length of the filaments. In figures 171 and 172 are shown
two views of a cymule of *A. nitida* with a tetramerous secondary
floret and a pentamerous tertiary floret. In *A. nepalensis* the tend-
ency toward trimery and dimery is also strongly expressed. The
vascular supply of each of these florets, as in other members of the
family, is derived from either side of the gap left by the departure
of the subtending secondary bract and therefore represents the
tertiary axis. It is significant that the florets at the distal end of the
ament in *A. nepalensis* often lack tepals, so that the florets are
represented by their stamens alone, which are reduced in number
to but one or two each.

In s. Cremastogyne, both species have the same type of floral
morphology, which is essentially like that described for the terminal
portions of the ament in *A. nepalensis*. The tepals are completely
absent (*A. lanata*, figs. 176–179) and but one stamen, *d*, remains
to represent each of the tertiary florets while the secondary floret

is represented by two stamens, x and x'. That three florets are present is indicated, not only by homology with the reduced cymules to be found in *A. nepalensis*, but also because each floret has its individual vascular supply which arises similarly to that in species where the florets are readily recognizable as distinct.

In this genus the whole gamut is run from hexamerous to mono-merous florets, as well as from the presence of a complete perigon to its complete absence. Where a perigon is present, the filaments of the stamens are fused with the tepals for an appreciable distance, although this is masked in s. Clethropsis by the extreme length of the free portion of the filaments.

Betula, staminate florets (for species studied see footnote 11)

While radial symmetry predominates in the individual florets of the preceding genus, in the florets of the various species of *Betula* there is characteristically a tendency toward zygomorphism and toward the presence of divided anthers. The zygomorphism is cor-related with the shortening of the secondary axis of the cymule, which crowds the individual florets against the primary axis of the ament. Associated with this pressure there is suppression of the floral parts toward the primary axis. This is least evident in avail-able material of *B. maximowicziana* (figs. 219, 220). The secondary floret often has the tetramerous plan completely carried out in both the perigon and the androecium, but in the example shown (fig. 219) the adaxial perigon segment is represented only by a gland-tipped protuberance. These protuberances are of considerable importance in the floret morphology of the family as a whole, since the margins of the tepals are regularly studded with these glands. In general these glands have been omitted from the habit sketches because they so complicate them, but an idea of their mode of distribution on the perigon segments may be gained from figure 181. The glands persist in their association with the tepal in all stages of its reduction, and since these glands are of a peculiar elongated type, differing from the flat lenticular ones commonly occurring on the surfaces of the bracts of *Alnus* and *Betula*, their presence in the absence of a perigon segment is considered to be indicative of the former presence of a tepal. The tertiary florets (fig. 220) of *B. maximowicziana* are

also often tetramerous but are more zygomorphic than are the secondary florets. Trimery also occurs commonly in the florets of this species, and is achieved by the loss of the adaxial stamen of the secondary floret or one of the diagonal stamens in the tertiary florets.

Trimery occurs commonly in the florets of species in ss. Costatae, although an occasional tetramerous floret is present as in *B. lenta* (fig. 181). Dimery becomes the rule in *B. nigra* of this section.

In the ss. Albae, while trimery sometimes occurs, dimery of the individual florets is the rule, and there is a strong tendency toward the reduction of the number of tepals to one in each floret, with the other tepals represented by the stalked glands previously referred to, as shown in *B. pendula* (fig. 198).

In the ss. Nanae trimery is very rare, being found only occasionally in cymules from the base of the ament in *B. glandulosa*. Dimery is fairly common in the secondary florets, while the tertiary florets are generally monomerous; but the secondary floret may also be monomerous (*B. pumila*, fig. 210).

B. alnoides var. *pyrifolia* and *B. luminifera* of ss. Acuminatae differ from the other species of *Betula* thus far mentioned in the reduction of the lateral members, y and y, of the secondary floret, and by the persistence of perigon segments in the absence of the stamens associated with them, a situation rather reminiscent of some species of s. Gymnothyrsus (fig. 174) of *Alnus* than of the other species of *Betula*.

Throughout the genus the vascular system supplying the florets is very simple. Oddly enough the more reduced tepals often lack a vascular system, although they may still be recognizably foliose, such as tepal c in figure 196. Even if supplied with vascular tissue, this may be entirely unconnected with the main vascular system of the cymule. There may well be a direct correlation here between extent of vascularization and absolute size of the organ.

The origin of the vascular supply to the members of the florets composing the cymule has been described under the cymule anatomy. Whether the florets are tetramerous or monomerous, the origin of their vascular system is basically the same. The chief difference is in the number of individual traces, which depends on the number of parts the floret might have. Well developed tepals

have a three-parted vascular supply after the departure of the sta-
men bundle in *Alnus*.

Carpinus, staminate florets (see footnote 12)

The bewildering array of stamens and the absence of tepals in
the cymules of this genus and in *Ostrya* have long been the occasion
of a misinterpretation of the true morphology of the cymule. This
confusion is increased by the division of the stamens extending far
down into the filament proper. Upon removal of the anthers, it may
be seen that the filaments tend to be arranged in three groups corre-
sponding to the secondary and tertiary florets. As pointed out in
the description of the vascular system of the staminate cymules in
this group, the vascular supply to the stamens is homologous with
that of other cymules in the family where three florets are obviously
present. The individual florets are often hexamerous (*C. japonica*,
fig. 237) in the species of s. Distegocarpus (sometimes up to 8-
merous), and as either end of the ament is approached may become
reduced to dimerous (*C. japonica*, fig. 242) or even monomerous
(*C. japonica*, fig. 235). The species of the other section have their
florets less often hexamerous, more commonly pent- or tetramer-
ous (figs. 232, 234), and even trimerous.

Ostrya, staminate florets (see footnote 7)

Morphologically the florets of this genus are identical for all
practical purposes with those of s. Eucarpinus of *Carpinus* with a
tendency toward a smaller number of stamens per floret.

Ostryopsis, staminate florets

The regular thing observed in the two species of this genus is
dimery of the secondary and tertiary florets (fig. 270), or dimery
of the secondary and monomery of the tertiary florets (fig. 268).
Otherwise the general condition is the same as that in *Carpinus* and
Ostrya.

Corylus, staminate florets (see footnote 8)

As in *Ostryopsis*, the florets are either monomerous or dimerous.
Tepals are also lacking. The stamens are completely divided to the
base. Sometimes, in the proximal portion of the aments, the stamen

halves of the tertiary florets may be rather widely separated from each other (fig. 255); ordinarily, however, they are closely associated (figs. 253, 257).

Occasionally small foliose structures are found in the position of tepal x' (fig. 253) or of x (fig. 257) which have diminutive vascular supplies. This indicates that these may be tepals. In teratological specimens, to be discussed in the next paper of this series, the complete perigon complement of the secondary floret may occur, which further suggests that these are vestigial perigon segments in the normal material.

As pointed out in the discussion of the vascular system of the cymule, the origin of the vascular supply to the monomerous or dimerous florets in this genus is such as to establish their homology with other less reduced ones.

PISTILLATE FLORETS: MORPHOLOGY AND ANATOMY

A striking diversity of external form characterizes the pistillate florets of the Betulaceae. In the genera of the Coryleae an easily recognizable perigon is present throughout, clearly confirming the fact that the ovary in this subfamily is inferior. But in the other subfamily, the Betuleae, although evidently closely related, no evidence of a perigon has been reported except by WOLPERT (36).

Ostrya, pistillate florets

The species studied in this genus (see footnote 7) possess the same general type of floral morphology as that shown for *O. virginiana* (fig. 135). The ovary is inferior owing to its fusion with a tetramerous perigon (*pg*, figs. 135, 136) which in the older stages is extended in a tubular form slightly beyond the body of the ovary. The vascular supply of the floret is derived from the dictyostele forming the tertiary vascular axis (*ped*, fig. 130). From this dictyostele a number of anastomosing branches (*anas*, fig. 131) progress radially inward to form the compound ventral bundle of the bicarpellary ovary, while the remaining portion of the dictyostele progresses upward into the perigon, forming the perigon supply (*pg*, figs. 131, 132). It is from members of this perigon supply that branches are derived to form the two dorsals of the ovary (*dr*, fig. 132). The dorsals arise

in the same plane as did the tertiary bract bundles, an aid in considering the orientation of the ovary. These dorsals continue upward throughout the length of the ovary and into the styles without further branching. The perigon supply passes with some branching into the perigon tube, while the ventral bundle divides laterally into two branches, each supplying an ovule (fig. 134). The division occurs in the septum separating the two loculi of the ovary. It is only after the ovule supply has departed that the septum disappears, permitting the formation of a fusion loculus. This loculus is continuous with the rather conspicuous interstylar canal and is a characteristic feature of the Betulaceae. Its presence is apparently correlated with the complete disappearance of the compound ventral bundle beyond the region of insertion of the ovules, so that there is no vascular tissue to support the loose parenchymatous tissue of the septum. The number of bundles supplying the individual tepal is variable, even within the same species or within the same cymule, but basically there seem to be three for each tepal as in the staminate florets of *Alnus*. With the close association of the tepals due to their lateral fusion, occasional adjacent perigon bundles have probably fused to form a compound trace.— a well known phenomenon in floral anatomy.

Carpinus, pistillate florets (see footnote 6)

These florets are very similar to those described for *Ostrya*, except that the perigon does not extend beyond the ovary. The tepals are generally represented simply as small teeth, as in *C. japonica* (fig. 139); although sometimes as in *C. caroliniana* (fig. 153) the individual teeth are three-lobed, lending support to the idea already expressed that the vascular supply to the tepals in this subfamily is basically tripartite. The vascularization of the florets is practically identical with that in *Ostrya*. The septum in the ovary disappears at the level of the division of the compound ventral bundle, which occurs slightly below the insertion of the ovules (fig. 150), giving the ovules the appearance of being parietal. This is, however, a secondary phenomenon. It is significant that sometimes it is the ovules of directly opposite margins of the two carpels that receive the vascular supply and sometimes it is the diagonally

opposite margins that are involved (fig. 150). It is rarely that all four ovules, one for each margin of each of the two carpels, develop. There does not seem to be any particular rule about which of the ovules develops in any given case. The supply to the ovules in these two genera is tripartite, with further branches arising from the two laterals in the base of the ovule. The dorsal bundles arise in the plane of the tertiary bract bundles, indicating that the same basic orientation exists in this genus as in *Ostrya*.

Ostryopsis, pistillate florets

In general the florets in this genus show a strong similarity to those in the preceding two genera, but the perigon tube is somewhat longer than its homologue in *Ostrya* and does not have such well defined teeth, but is nevertheless obviously tetramerous (*pg*, fig. 99).

The vascular supply of the florets is complicated by the shortness of the tertiary axis, so that the floral bundles depart shortly after the departure of the tertiary bract bundles (fig. 106). Nevertheless essentially the same type of floral supply exists here as in *Carpinus* and *Ostrya*. The tepal bundles differentiate immediately after the departure of the tertiary bract bundles; the ventral bundle forms by anastomoses from among the perigon bundles; and the dorsals arise as branches of the perigon bundles. The compound ventral bundle divides in the septum and supplies the two ovules, after which the septum breaks down, permitting the formation of a fusion loculus.

The dorsals arise in the plane of the abaxial tertiary bract bundle and that of the secondary bract bundle, so that the ovary is diagonal to the secondary bract.

Corylus, pistillate florets (see footnote 8)

The perigon of the florets in all the species studied in this genus is so reduced that it is recognizable only as an irregularly sinuate fringe (*pg*, figs. 109, 119, 122). It is difficult to assign a definite number to the tepals which compose the perigon, although it is probably four. The vascular system of the floret proper is much dissected because of the many anastomoses from the tertiary vascular axis (fig. 112) which form the supply to the tertiary bracts. An

inner set of anastomoses arises simultaneously to form the small cylinder responsible for the formation of the ventral bundle. From this cylinder a third set of anastomoses again passes outward to refuse with traces persisting after the departure of the tertiary bract bundles to form the perigon supply. Because of the discrete nature of the supply to the tertiary bracts and the absence of the secondary bracts it is difficult to assign a definite orientation to the ovary, but from a study of the younger stages, before increase in size of the vascular system, it seems clear that the ovary is diagonal.

The ventral bundle divides somewhat below the insertion of the two ovules, which are on diagonally opposite margins of the two carpels in this ovary. The number of traces in the perigon is much greater than it would be if each of the perigon segments were supplied with but three bundles. This may be explained by the extreme dissection of the vascular system in the much abbreviated tertiary axis and the great increase in size of the ovary over that usual in the family. Thus a greater degree of dissection of the vascular supply to the individual tepals than that observed elsewhere in the family may have occurred.

Alnus, pistillate florets (see footnote 2)

The individual florets ("pistils") in this genus present a delusive appearance of simplicity. Examples of these from widely differing species are shown in figures 38 and 44. An occasional species such as *A. subcordata* (fig. 41) has as appendages to the small flattened "pistil" two or more glands at the base of the stylar column. These glands are especially well developed in this species, the presence of four being unusual for the genus, although common in this species. The glands uniformly occur one opposite each of the dorsals of the ovary and one on either side opposite the septum. In other words, these glands are in the positions which would be occupied by tepals in a tetramerous perigon. Furthermore, minute but recognizable vascular bundles are sometimes to be found in the outer portion of the ovary wall in the positions they would normally occupy if there were four such perigon segments fused to the ovary. In most species of the genus these glands are much smaller and opposite the dorsals only, or may be absent completely. However, in one lot of material

of *A. rubra*, otherwise normal, there are definite free or partially fused tepals. In this case, which will be further described in the next contribution of this series, vascular bundles are regularly associated with the partially free tepals. On the other hand, in *A. incana*, with only small glands or none at all, occasional vestigial bundles are present in the basal portion of the ovary, bundles which are evidently homologues of those supplying the poorly developed or vestigial perigon segments of other species.

Aside from the weakly developed or completely suppressed tepal supply, the vascular system of the florets is simple. The traces composing the tertiary vascular system combine loosely to form a diminutive pedicellar siphonostele (figs. 13, 30, 55) from which depart almost immediately the two dorsal bundles (figs. 14, 30, 57). The remaining vascular tissue combines to form the ventral fusion bundle (figs. 31, 58). In several species this bundle divides into two pairs of three bundles each (*A. crispa* var. *mollis*, fig. 31; *A. incana*, fig. 61), each set of three supplying an ovule. Beyond this point there is no extension of the ventral bundle. The septum breaks down immediately after the departure of the vascular tissue to the ovules (figs. 33, 34), forming a fusion loculus (figs. 34, 35, 62) which is continuous with the interstylar canal. On the whole the vascular system of the ovary is identical with that of the other members of the family, except for the drastic reduction of the perigon, correlated doubtless with the great reduction in size of the floret.

The ovaries at first appear to be median to the secondary bracts but upon closer observation are clearly diagonal, slightly modified by the pressure of adjacent cymules.

Betula, pistillate florets (see footnote 5)

Morphologically the florets of this genus are much like those of *Alnus*. No cases were found of tepals as well defined as they are in some species of *Alnus*. Glands similar to those present in some species of *Alnus* also appear sporadically in this genus, as in *B. coerulea-grandis* (fig. 98′) and in *B. maximowicziana*. In no case have vascular elements been found associated with these glands as in some species of *Alnus*, there being present only elongate hyaline rows of cells approaching the insertion of the gland at the base of

the stylar column. The homology of the glands in *Alnus* and *Betula* is shown by their great similarity, indicating that in *Betula* these glands also suggest the former presence of foliose tepals.

The vascularization of the florets is also much like that of the florets in *Alnus* although somewhat simpler. There is much greater reduction of the vascular tissue composing the tertiary vascular axis, and it is not cylindrical as in *Alnus*. Thus there is simply a branching of the vascular tissue to make up the dorsals on the one hand and the ventral on the other. The dorsals follow the usual course, as does the ventral. In the case of the ventral, however, it simply bifurcates to supply the two ovules, which may be on adjacent or diagonally opposite carpel margins. The septum of the ovary does not ordinarily break down until after the departure of the ovule bundles, and there follows the formation of a fusion loculus, ultimately continuous with the interstylar canal. Occasionally tricarpellary ovaries occur, as in *B. luminifera* (fig. 86).

The tertiary florets have their ovaries "medianly" (diagonally) oriented with respect to the secondary bract, while the secondary floret has its ovary transverse to the primary bract.

SUMMARY OF FLORET MORPHOLOGY AND ANATOMY

It is clear from the uniform presence of a perigon in the pistillate florets of the Coryleae, and the occurrence of a perigon in the staminate florets of the Betuleae, that the members of the family must be considered as derived from some ancestor which possessed perigon segments regularly in the florets of both sexes. Furthermore, vestiges of a perigon are found occasionally in the staminate florets of the Coryleae and in the pistillate florets of the Betuleae, suggesting reduction of the perigon in these forms.

In the less reduced staminate florets a hexamerous condition is characteristic, while in the pistillate florets trimerous pistils occur in almost every genus. If these indications are significant then we must hypothecate for the family as a whole an ancestral condition in the phylogenetically recent past in which the florets were trimerous throughout, as shown in the floral diagrams in figures 5 and 6. Under such circumstances we are in a position to evaluate the puzzling differences in orientation of the pistils in the various genera of

the family. The diagonal orientation of the pistil in the tertiary pistillate florets of *Ostryopsis* (fig. 100), *Alnus* (fig. 39), and *Betula* (fig. 77) may well be associated with the suppression of the adaxial diagonal carpel of a tricarpellary ovary (fig. 5). On the other hand the transverse pistils of *Carpinus* (fig. 152) and *Ostrya* (fig. 123) are doubtless associated with the suppression of the median carpels of the tertiary florets (fig. 5). There remains the condition in *Corylus* (fig. 120) in which the ovaries of the tertiary florets appear to be median to the secondary bracts, but this seems to be associated with a slight displacement of ovaries originally diagonal.

In the pistillate cymules of *Betula* there is the apparent anachronism of diagonal pistils in the tertiary florets and a transverse pistil in the secondary floret (fig. 77). This, of course, may be explained either by the suppression of the median carpel in the secondary floret and also of the adaxial carpel in the tertiary florets, or by the suppression of one of the diagonal carpels in the secondary florets and a later twisting of the floret to bring the pistil into a transverse position. The answer to this problem awaits the solution of the question whether the florets of *Betula* became dimerous after the effects of crowding in the ament had been reflected in the morphology of the florets, or whether this reduction to dimery came earlier. Either point of view is tenable.

In the staminate florets there is a remarkable parallel series in the reduction of the androecium in *Alnus* and *Betula* on the one hand and in *Carpinus*, *Ostrya*, *Ostryopsis*, and *Corylus* on the other hand. In the former the reduction series is accompanied by a corresponding reduction in the perigon and in the latter by a complete absence of the perigon, with the possible exception of *Corylus*. In both series there is also a tendency toward separation of the anthers, a tendency which goes much further in the latter series than in the former.

Literature review

The following significant questions arise in reviewing the literature on the morphology of the inflorescence and florets.

I. INTERPRETATION OF PRIMARY, SECONDARY,
AND TERTIARY BRACTS

Up to the time of HARTIG (1852) and DÖLL (1843) the bracts were considered as simply scales or appendages, or were even confused with tepals, although the latter misconception was early recognized as such by FANT (12). With the increasing tendency to homologize the foliar organs with one another under the influence of the metamorphosis theory of GOETHE (13), two schools of thought arose, the one founded by HARTIG (15) and the other by DÖLL (8). HARTIG's stipular theory to account for certain of the bracts has been variously modified and finds a recent exponent in PAUCHET (20). On the other hand, DÖLL's (9) theory of the strictly laminar nature of the different bracts has attracted a tremendous following (22, 11, 23, 1, 35, 36, 26, and most other contemporary morphologists and systematists). Since, however, the stipular theory is so ingenious in certain of its forms (33), it is well to review the anatomical evidence against it. A single example will suffice. In the staminate cymules of *Carpinus japonica* it was found that those from the base of the ament had, instead of primary bracts, a reduced lamina flanked by stipules (fig. 241), and that secondary bracts were present as well. The vascular supply to these stipules is derived as branches departing laterally from the laminar supply subsequent to its becoming free from the primary vascular cylinder. The vascular supply to the secondary bracts is derived from the secondary axis cylinder in the usual fashion, and is distinct in every way from that to the reduced lamina and its stipules. Passing to a cymule in which the primary bract is typically developed and is accompanied by secondary bracts (fig. 239), the superficial resemblance of this complex of bracts suggests that the secondary bracts may represent the stipules of a reduced leaf of which the primary bract represents the lamina. That this is not the case is evidenced by the complete lack of relationship between the vascular supply of the primary and the secondary bracts, since the primary bract derives its supply from the base of the gap in the primary vascular axis while the secondary bracts receive their vascular supply from bundles departing laterally from the secondary vascu-

lar axis. Had these secondary bracts been stipules, as the adherents of the stipular school would hypothecate, then their vascular supply would have consisted of branches from the primary bract bundle. Throughout the family, in the modified leaves entering into the composition of the cymule there is no evidence of deviation from the anatomical pattern characterizing the vascular origin of successive exstipulate leaf organs on a shortened axis. DÖLL's theory is fully substantiated by the anatomical evidence.

The absence of secondary bracts in the pistillate cymules of *Corylus* has been noted by only two workers, SCHACHT (27) and VAN TIEGHEM (33). That this important fact has been overlooked is doubtless due to the superficial resemblance between the tubular involucre of some species of *Corylus* and that of *Ostrya*, which because of its evident close relationship to *Carpinus* has been considered to have both secondary and tertiary bracts. SCHACHT and VAN TIEGHEM found, by careful ontogenetic study of species of *Corylus* in which the involucre is composed of free members, that three bract primordia were present in the early stages of development, but that the one occupying the position of the secondary bract very early ceased developing while those in the position of the tertiary bracts persisted to form the involucre. The vascular system of the cymule has been considered in detail in this paper and substantiates the conclusions reached earlier by SCHACHT and VAN TIEGHEM.

2. NATURE OF STAMINATE INFLORESCENCE IN THE CORYLEAE (CARPINUS, OSTRYA, CORYLUS, AND OSTRYOPSIS)

Almost without exception the staminate ament of the Coryleae has been interpreted as a spike, the implication being that each primary bract subtends but a single floret. As early as 1851, however, WYDLER (37) suspected that the staminate ament in this group was composed of a series of cymules, since he found the number of stamens in each cymule to be about three times as many as they were in each of the three florets of a corresponding cymule in the Betuleae. Furthermore he pointed out the divided nature of the stamens in the Coryleae, which helped clarify the morphologi-

cal concept of the time. His idea was put forward only as a sugges-
tion without proof, a form in which EICHLER (11) gave it his sanc-
tion. The concept has lain dormant but is evidently in need of re-
vival because the vascularization of the cymules indicates clearly
the correctness of WYDLER's intimation.

3. NATURE OF THE OVARY: AXIAL OR APPENDICULAR

Many workers from the time of SCHACHT (27) have recognized the
foliar nature of the stigmas of the pistils of the Betulaceae, but
interpretation of the nature of the ovary wall has been varied. Thus
SCHACHT, following PAYER's (21) general line of reasoning, conclud-
ed that the ovary wall in the Betulaceae is axial, a point of view
adopted in a modified form by NAVASHIN (19) and STREICHER (31).
Opposed to this interpretation of the ovary wall in flowering plants
in general are VAN TIEGHEM (32), HENSLOW (16), and EAMES (10).
The latter two consider the ovary wall to be foliar even though it
may arise as it does in the Betulaceae through the intercalary
growth of a ring of tissue rather than from as many distinct "an-
lagen" as there are carpels in the ovary. In no way does the vascular
anatomy of the ovaries of the Betulaceae support the notion that
either the walls or the placentae are axial, since the vascular supply
to the carpel walls arises at the base of the ovary in a manner like
that of a reduced leaf and bears no similarity in behavior to the
vascular tissue in stems. Thus the arguments brought forward by
WOLPERT (36) in favor of the appendicular nature of the ovary
and placentae in this family are fully supported by the anatomical
evidence.

4. SUPERIOR OR INFERIOR CONDITION OF OVARY
IN THE BETULEAE

The great majority of the students of the Betulaceae have
accepted the ovary of the species in the subfamily Betuleae without
question as being superior, or when in doubt, have referred to it as
nude (BENTHAM and HOOKER 3). DÖLL (8), and later WOLPERT
(36), have suggested that the ovary in this group has fused to it a
rudimentary perigon. They employ one of the four lines of evidence
which lead to this conclusion. This is the presence at the base of the

stylar column of glandular structures resembling those present on
the margins of the tepals in the staminate florets (fig. 41). Other
lines of evidence of which they did not avail themselves are the
following. First, in the ontogenetic field, where it has long been a
matter of common knowledge that the inferior ovary is distinguished
from the superior ovary by arising through the basal growth of a
ring of tissue (21, 7). In the Betuleae the ovary wall forms as a result
of the basal growth of a ring of tissue (19), evidence that the ovary
is essentially inferior. Second, as has been pointed out in the pre-
ceding pages, occasional vestigial bundles occur in the walls or at
the base of the ovary in species of *Alnus*. These bundles are in the
position occupied by the major bundles of the perigon in genera of
the Betulaceae where a perigon is undoubtedly present. Third,
there has been observed in a single collection of *A. rubra* a perigon
composed in part of free and in part of fused tepals. While this may
be considered teratological, and therefore perhaps of secondary
importance, the other three lines of evidence support the validity
of this particular evidence. While it is not intended to state dog-
matically that a perigon is present in all species of the Betuleae,
for it is not reasonable to say that a structure is present if it has been
lost, it is nevertheless suggested that the pistil of the Betuleae be
recognized as inferior, and representative of a much reduced floret.
The only other interpretation possible is that the perigon is arising
anew, an idea unacceptable in the light of Dollo's law.

5. MISCONCEPTIONS AND UNSOLVED PROBLEMS

Perhaps the most striking misconception is that concerning the
morphology of the florets in the species of s. Clethropsis in *Alnus*.
SPACH (29) described the staminate cymules as uniflorous with 10- to
12-parted florets. The idea has persisted in the literature essentially
unchallenged except by EICHLER (11), who suggests that there may
be a three-flowered cymule here. That this is actually the case has
been pointed out in the foregoing pages. The error was doubtless
due to the discrepancy in size between the diminutive tepals and the
long-filamented stamens, together with the paucity of the material,
since SPACH (29) made a similar error in the interpretation of the
cymule of the species of s. Alnobetula of *Alnus*. In the latter case an

abundance of flowering material was readily available in Europe, and the mistake was corrected by SPACH's successors.

The amazing suggestion made by DE CANDOLLE (4) that the florets of the staminate cymules in the Betulaceae are simply proliferations on the upper surface of the complex of bracts deserves comment primarily for its eccentricity, and indicates to what extremes a purely ontogenetic study may lead. If the concept of the flower as a short shoot is to be abandoned, as VUILLEMIN (34) and GRÉGOIRE (14) would have us believe, and if the inflorescence is not a branch system as DE CANDOLLE implies, then there is no sound basis left for the study of comparative floral and inflorescence morphology.

A major problem, first recognized by DÖLL (8), is the variation in the orientation of the pistil from genus to genus. Two factors are to be considered. One is the effect of the vigorous crowding together of organs in the ament. Doubtless it is this which is responsible for the absence of the secondary florets in all the genera of the family except *Betula*. In *Betula* this factor may be responsible for orientation of the secondary floret ovary transverse to the primary bract whereas the ovaries of the tertiary florets are roughly median to the secondary bracts. For conclusive evidence we must resort to experiment, but, in the absence of this, the circumstantial evidence to be derived from the presence of secondary florets in the proliferated material of *Alnus* is instructive. Here it is found that in the absence of a strong dorsiventral pressure the secondary floret has its ovary disposed medianly to its bract, thus indicating, by analogy at least, the effect of absence of pressure on the orientation of the ovary of the secondary floret.

The second factor involved in a consideration of the orientation of the ovaries is that dealing with the occasional presence of tricarpellary ovaries in the family. While this will be described at greater length in the next paper of this series, it suffices to state that in every genus studied tricarpellary ovaries have been found in one or more species, often occurring in otherwise normal aments. In the light of Dollo's law it is not unreasonable to suppose that tricarpellary ovaries were once more prevalent in the florets of the family than they are today. Many observers from the time of

SPACH (30) have noted the occasional presence of tricarpellary ovaries. Furthermore, supporting evidence is to be derived from the staminate florets, since they are often hexamerous; and when not hexamerous, WOLPERT (36) has pointed out that these florets may begin with the *anlagen* of a hexamerous condition, only to have some of the *anlagen* suppressed in later development. Granting the possibility that the trimerous gynoecium (floral diagram in fig. 5) was ancestral and the dimerous gynoecium derived, there is the further possibility that the tertiary florets have become bicarpellary by the loss of either the carpel in the plane of the secondary bract or one of the carpels in the plane of the tertiary bracts. In *Carpinus*, *Ostrya*, and *Ostryopsis* the vascularization of the gynoecium indicates that the carpel in the radius of the abaxial tertiary bract is present, because its dorsal arises in the same radius as does the bundle to the abaxial tertiary bract. The dorsal of the other carpel, however, arises in *Carpinus* and *Ostrya* in the radius of the adaxial tertiary bract trace, while in *Ostryopsis* it arises in the radius of the secondary bract trace. The result is the orientation of the ovary transverse to the secondary bract in *Ostrya* and *Carpinus*, and a "median" (or more properly, diagonal) orientation of the ovary to the secondary bract in *Ostryopsis*. In *Alnus*, *Betula*, and *Corylus* the changes imposed by extreme smallness or largeness of the ovaries on the vascular systems have obscured the relationships of the dorsals of the ovaries to the bract traces; but it is evident that essentially they share with *Ostryopsis* the diagonal orientation of the ovaries, which has erroneously been considered median to the secondary bracts.

The staminate florets in the Betuleae provide no problem in interpretation, since forms which are reduced to monomery, such as those which occur in the ss. Nanae of *Betula* or in the s. Cremastogyne of *Alnus*, are connected by a continuous series of intermediate stages with morphologically orthodox hexamerous florets. They are also provided with vascular systems whose characteristics are in accord with the preceding interpretation.
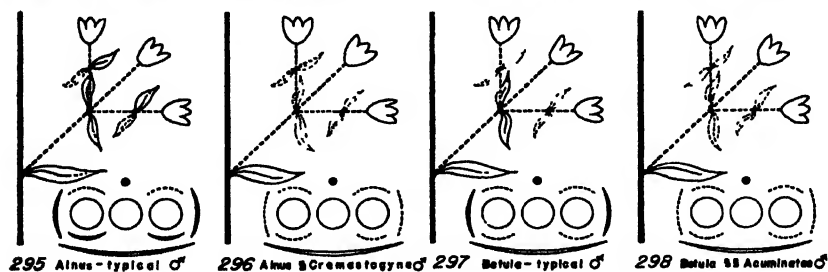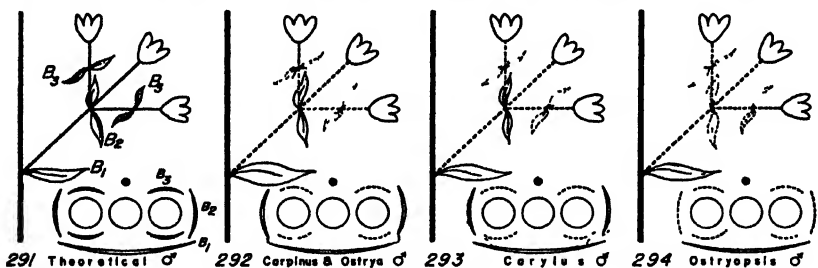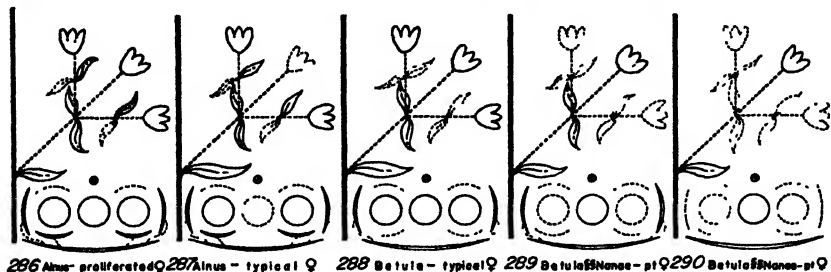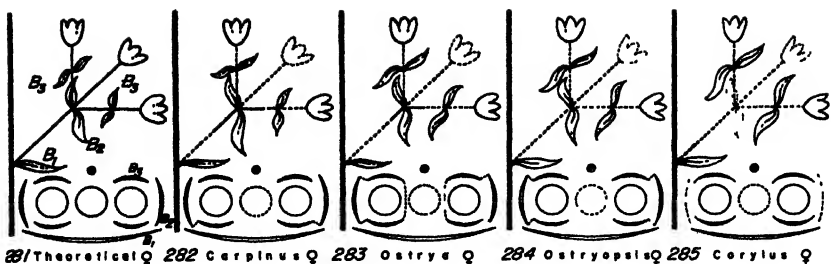
FIGS. 281-298.—Figs. 281-290, diagrams to show presence (solid lines), absence (broken lines), and fusion (dotted lines) of components of more common types of pistillate cymules in Betulaceae. Figs. 291-298, diagrams to show presence (solid lines), absence (broken lines), and fusion (dotted lines) of components of more common types of staminate cymules in Betulaceae.

Summary

1. The anatomical method provides a key to the interpretation of floral and inflorescence morphology in the Betulaceae. The floret has the anatomical characteristics of a short shoot and undoubtedly is of that nature. Likewise on an anatomical basis the inflorescence is a branch system.

2. The morphology and anatomy both of the cymules and of the florets have been notably modified by dorsiventral or lateral concrescence, shortening of internodes, pressures within the ament, and reduction.

3. The bicarpellary ovaries, either transverse or diagonal to the secondary bract, owe their diverse orientation to their derivation from an ancestral tricarpellary ovary. The staminate floret in many species still has a trimerous groundplan.

4. On the basis of these factors, the morphology of the cymules and florets of the Betulaceae may be interpreted (figs. 281-298) as follows:

a. The staminate cymule is three-flowered in all genera of the family. The pistillate cymule is two-flowered by suppression of the secondary floret in all genera except *Betula*, in which all three florets are present.

b. The full complement of bracts is present in the pistillate cymules of *Carpinus*, *Ostryopsis*, and *Ostrya*. The adaxial tertiary bract is lost from the staminate and pistillate cymules of most species of *Alnus*. Both adaxial and abaxial tertiary bracts have been lost from the staminate cymules of *Betula*, *Corylus*, *Carpinus*, *Ostrya*, and from the pistillate cymules of *Betula*. The secondary bracts have been lost while the tertiary bracts persist in the pistillate cymules of *Corylus*. All bracts but the primary have been lost in the staminate cymules of *Ostryopsis*.

c. The perigon is present in the pistillate florets of *Carpinus*, *Ostrya*, *Corylus*, *Ostryopsis*, and in the staminate florets of *Alnus* and *Betula*; it is obsolescent in the pistillate florets of *Alnus* and *Betula*; it is completely lost from the staminate florets of *Carpinus*, *Corylus*, *Ostrya*, and *Ostryopsis*. The ovary is inferior throughout the family.

d. The phylogenetic loss of the carpel in the radius of the second-ary bract has resulted in the transverse bicarpellary ovary of *Carpinus* and *Ostrya*. The loss of the carpel in the radius of the adaxial tertiary bract has resulted in the so-called "median" (or more properly, diagonal) bicarpellary ovary of *Alnus*, *Betula*, *Corylus*, and *Ostryopsis*.

LITERATURE CITED

1. BAILLON, H., Natural history of plants. Transl. by M. M. HARTOG. London. 1871.
2. ———, Récherches organogéniques sur les Amentacées. Assoc. Franc. Avanc. Sci. Confs. (1875):756–771. 1875.
3. BENTHAM, G., and HOOKER, J. D., Genera plantarum. Vol. III. London. 1883.
4. de CANDOLLE, C., Sur les bractées florifères. Bull. Herb. Boisser 1:124–127. 1893.
5. CHAMBERLIN, T. C., The method of multiple working hypotheses. Jour. Geol. 5:837–848. 1897.
6. CHESTER, K. S., ABBE, E. C., and VESTAL, P. A., Studies in the "precipitin reaction" in plants. V. Application to plant relationships. Jour. Arnold Arb. 14:394–407. 1933.

7. COULTER, J. M., On the appearance of the relation of ovary and perianth in the development of Dicotyledons. BOT. GAZ. 10:360–363. 1885.

8. DÖLL, J. C., Rheinische Flora. Frankfurt a. M. 1843.

9. ———, Zur Erklärung der Laubknospen der Amentaceen. Frankfurt a. M. 1848.

10. EAMES, A. J., The vascular anatomy of the flower with refutation of the theory of carpel polymorphism. Amer. Jour. Bot. 18:147–188. 1931.

11. EICHLER, A. W., Blüthendiagramme. Vol. II. Leipzig. 1878.

12. FANT, E. M., Betula. Dissertation, Upsala. 1807.

13. GOETHE, J. W., Versuch die Metamorphose der Pflanzen zu Erklären. 1790. (Transl. by E. M. Cox. Jour. Bot. 1:327–345; 360–374. 1863.)

14. GRÉGOIRE, V., La valeur morphologique des carpelles dans les Angiospermes. Acad. Roy. Belgium, Bull. Cl. Sci. 17:1286–1302. 1931.

15. HARTIG, T., Vollständige Naturgeschichte der Forstlichen Culturpflanzen Deutschlands. Berlin. 1852.

16. HENSLOW, G., The origin of floral structures. London. 1888.

17. JACKSON, G., Crystal violet and erythrosin in plant anatomy. Stain Technol. 1:33–34. 1926.

18. MASSART, J., La récapitulation et l'innovation en embryologie végétale. Bull. Soc. Roy. Bot. Belgique 33:150–241. 1894.

19. NAVASHIN, S., Kurzer Bericht meiner fortgesetzten Studien über die Embryologie der Betulineen. Ber. Deutsch. Bot. Ges. 12:163–169. 1894.

20. PAUCHET, L., Récherches sur les Cupulifères. Thesis, Paris. 1909.

21. PAYER, J. D., Traité d'organogénie de la fleur. Paris. 1857.

22. ———, Observations sur les fleurs mâles du Bouleau. Bull. Soc. Bot. France 5:151. 1858.

23. PRANTL, K., Beiträge zur Kenntniss der Cupuliferen. (ENGLER'S) Bot. Jahrb. 8:321–336. 1887.

24. ———, Betulaceae. In ENGLER and PRANTL, Die Natürlichen Pflanzenfamilien. Part I. 3:38–46. 1889.

25. REHDER, ALFRED, Manual of cultivated trees and shrubs. New York. 1927.

26. RENDLE, A. B., The classification of flowering plants. Vol. II. Cambridge, England. 1925.

27. SCHACHT, H., Der Baum. Berlin. 1853.

28. SINNOTT, E. W., The anatomy of the node as an aid in the classification of Angiosperms. Amer. Jour. Bot. 1:302–322. 1914.

29. SPACH, E., Revisio Betulacearum. Ann. Sci. Nat., Bot. sér. 2. 15:182–212. 1841.

30. ———, Notes sur les Corylus. Ann. Sci. Nat., Bot. sér. 2. 16:98–108. 1841.

31. STREICHER, M., Zur Entwicklungsgeschichte des Fruchtknotens der Birke Denkschr. Akad. Wiss. Wien. Math.-Natwiss. Kl. 95:355–367. 1918.

32. VAN TIEGHEM, PH., Récherches sur la structure du pistil et sur l'anatomie comparée de la fleur. Mem. Sav. Acad. Sci. Inst. Imp. France 21:1–261. 1875.

33. ———, Rémarques sur la fleur fémelle des Chaunes, des Aunes et des Pacaniers. Ann. Sci. Nat., Bot. sér. 9. 3:369–374. 1906.

34. VUILLEMIN, P., L'Amphigonelle et la phylogenie des Amentales. Ann. Sci. Nat., Bot. sér. 10. 1:139–200. 1919.

35. WINKLER, H., Betulaceae. Das Planzenreich. IV. 61. 1904.

36. WOLPERT, J., Vergleichende Anatomie und Entwicklungsgeschichte von *Alnus alnobetula* und *Betula*. Flora 100:37–67. 1910.

37. WYDLER, H., Ueber die symmetrische Verzweigungsweise dichotomer Inflorescenzen. Flora 34:289–301; 305–312; 321–330; 337–348; 353–365; 369–378; 385–398; 401–412; 417–426; 433–448. 1851.

38. ZIRKLE, C., The use of N-butyl alcohol in dehydrating woody tissue for paraffin embedding. Science 71:103–104. 1930.

INFLUENCE OF SULPHUR DEFICIENCY ON THE METABOLISM OF THE SOY BEAN

Scott V. Eaton

(WITH TWO FIGURES)

Introduction

In an earlier publication (7) attention was called to the rather peculiar position of sulphur in the list of elements essential to the growth of plants. Ever since the now obsolete ten-essential-elements concept was established, sulphur has been a member of the list, although it has not been considered necessary to use sulphur fertilizers. One of the main reasons for this has probably been that the early ash-analysis method of determining sulphur in plants showed such a small amount present that it was thought that the supply in the soil plus that brought down by the rain was ample for the needs of crops. When later accurate fusion methods of analysis showed much more sulphur present than the ash analysis indicated, the whole question of the need of fertilizing crops with sulphur was reopened.

Most of these early investigations were carried on by agricultural experiment station workers, who were primarily interested in the practical aspects of the question. Numerous papers appeared dealing with such subjects as the sulphur content of soils, the amount of sulphur brought down by the rain, the sulphur content of crops, and the amount of sulphur in drainage water. But it was recognized that, after all, the best way to determine whether an element is deficient in the soil is to employ fertilizers containing this element and to see whether the yield is thereby increased. A number of workers investigated this question in connection with sulphur.

Of the agricultural soils of the United States, those of the northwest have been found especially deficient in sulphur (32, 26). A later contribution to the soil sulphur literature is that of POWERS (30), and recently STOREY and LEACH (34) showed that the disease of the tea bush known as yellows, which has seriously affected the growing

of tea in Nyassaland, Africa, is due to a deficiency of sulphur in the soils of this region.

Most of the investigations on sulphur having to do with plants have dealt primarily with its effect on crop yield, although some of the external effects have been noted, such as effects on color, size of plant, root development, and nodule development of the roots (**26, 32**). Also, some noted a higher nitrogen content of plants fertilized with sulphur (**26, 32**) and some divided the total sulphur of the plants into fractions (**20, 32**). But there has been lacking a thorough study of the effects of sulphur deficiency on the metabolism of the plant, such as has been supplied for the tomato by the recent work of NIGHTINGALE (**22**).

Before NIGHTINGALE's paper appeared the writer had started investigations on the effects of sulphur deficiency on the growth of plants. Preliminary experiments were performed in the spring of 1930. Most of this early work was concerned with the choice of a plant, several kinds being grown in washed quartz sand, including soy bean, alfalfa, rape, kale, mustard, wheat, and oats. Soy bean was finally selected.

Methods

GROWING THE PLANTS

PRELIMINARY EXPERIMENTS WITH SOY BEAN.—Considerable difficulty was experienced in demonstrating marked sulphur deficiency in the soy bean. The chief trouble was that the plants blossomed before any decided symptoms of sulphur deficiency appeared, and when the soy bean blossoms vegetative growth largely ceases. In the spring of 1932 Wisconsin Black soy bean plants were grown in pure quartz sand. Some of the plants were given a complete nutrient solution and some a solution lacking sulphates. The first blossoms appeared on June 20, after about four weeks of growth. The minus sulphur plants showed some symptoms of sulphur deficiency but they were not very marked. The plants were harvested on June 24 and analyzed for various carbohydrate and nitrogen fractions. Since the differences between the plus and minus plants were small, the results are not given.

It was decided to try to prolong the vegetative period so that the

effects of omitting sulphates from the nutrient solution would be greater. This was first attempted by increasing the nitrate content of the nutrient solution. In the fall of 1932 three sets of soy bean plants were grown in quartz sand with a nutrient solution the nitrate content of which was low in one set, medium in another, and high in the third. Each set was divided into two groups, one group receiving sulphate and the other receiving none. The vegetative period was prolonged somewhat by this treatment, but not markedly. The plants blossomed at about the normal time and again before marked symptoms of sulphur deficiency were obtained, although the minus sulphur plants were somewhat less green and had somewhat smaller leaves and thinner stems than the plus sulphur plants. It was perhaps hardly to be expected that the vegetative period would be much prolonged by this treatment, because of the short days of the autumn, many of which were cloudy. ECKERSON (11) has shown that poor light conditions cause a low reducase content of the Biloxi soy bean. Increasing the nitrate content of the nutrient solution under these conditions has little effect in increasing the growth of the plant or in prolonging the vegetative period, for the nitrates are not being used. The results might be different in the spring.

Attempts were next made to keep certain groups of plants in each of the nitrate series free of buds by picking them off as fast as they appeared. This was not very successful. Removing the buds tended to prolong the vegetative period somewhat, and the minus sulphur plants showed to a certain degree some of the characteristic symptoms of sulphur deficiency. But removing the buds caused them to be formed in even greater profusion, so that it was impossible to keep the plants entirely free of buds. Under these conditions uniform material for analysis could not be obtained.

It was found in previous work (8) that if the natural day length was prolonged to about 16 hours by using electric lights, soy bean plants did not blossom in about 5½ weeks. It was decided to use this method, although it was recognized that it was subjecting the plant to somewhat abnormal conditions.

FINAL PLAN.—Soy bean plants of the Manchu variety were grown in pure no. 3 quartz sand in 2-gallon glazed earthenware crocks provided with a hole in the bottom for drainage. The seed was given to

the writer by Dr. E. W. HOPKINS, who received it through the
kindness of the Department of Agronomy of the University of Wis-
consin. The sand was washed thoroughly with distilled water. The
plants were grown without nodule development, and to insure that
no nodules would develop the seed was sterilized with 0.25 per cent
uspulun solution and the sand was sterilized in a soil sterilizer. The
seeds were selected for uniformity, and an excess of seeds was
planted directly in the sand. About two weeks after the seeds had
sprouted, the seedlings were thinned to five uniform plants per pot.
Four 1000 watt Mazda bulbs were placed about 2 feet above the
plants. When daylight became somewhat weaker in the afternoon
these lights were turned on and left on until 10 P.M. The plus sulphur
series of 1933 consisted of 187 plants and the minus sulphur, 190; the
numbers in the 1934 experiment were 104 and 106 respectively.

The plants were given a nutrient solution that had been found in
previous work (8) well suited for the growth of the soy bean. Cer-
tain modifications were made for this work. Since the plants were
grown without nodule development, the nitrate content was higher
than in the previous work. Solution A of the formula consisted of
molecular calcium nitrate. In the case of the solution lacking sul-
phate, magnesium chloride in such an amount as to give the same
amount of magnesium was substituted for the magnesium sulphate.
The plants grown in the spring of 1934 received a somewhat more
concentrated nutrient solution during the latter part of the growth
period, when they were larger. The pH of the nutrient solution used
in 1933 was not controlled; that used in 1934 had an initial pH of
4.6- 4.9. The nutrient solution was sprinkled on the surface of the
sand. It was applied five days each week, enough being added so
that a considerable amount ran out through the hole in the bottom
of the crock. On the other two days of each week the sand was
thoroughly flushed with distilled water.

It was found necessary to supply the plants with a little boron.
A number of the plants grown in 1933 had dead tips after about six
weeks' growth. Dr. NIGHTINGALE, who was working in our labora-
tory at the time, said that this characteristic injury was no doubt
due to boron deficiency (2, 19). As soon as the injury was noted
boron was added twice each week at a concentration of 0.5 p.p.m.,

and as an additional precaution manganese was also applied at a concentration of 0.25 p.p.m. They were added with the distilled water used to flush the sand. In a short time shoots were developed from the axillary buds. In the case of the 1934 experiment, boron and manganese each at a concentration of 0.25 p.p.m. were applied twice each week throughout the period of growth, and no boron injury developed.

The soy bean plants were grown in the greenhouse in the springs of 1933 and 1934. It was impossible to control accurately the temperature and humidity. There was, of course, considerable variation in these conditions. In 1933 the seed was planted on March 7. Two samplings were made, one on April 22 and the other on May 5. In 1934 the seed was planted on March 1 and the plants were harvested on April 21. In 1934 sunflower, white mustard, kale, and rape were also grown, using the same nutrient solution and method that were used in growing the soy beans. The seed was planted on February 26 and the plants were harvested on April 27. No analyses were made.

CHEMICAL AND MICROCHEMICAL METHODS

SAMPLING AND EXTRACTION.—The method of sampling and extraction was essentially that described in a previous publication (8), except that in the present work leaves, stems, and roots were sampled separately, and the material was cut into fine pieces (21) instead of being ground in a mill. In the case of the plants grown in 1934, a composite sample of the stem tips, petioles, and axillary buds was made, so that the chemical composition of the entire plant could be estimated. The samples were extracted with hot 80 per cent alcohol, the alcohol being boiled each time on the steam bath for 15 minutes. The extract was allowed to cool to room temperature before filtering. Four or five extractions were made. It would probably have been better to have used a somewhat lower concentration of alcohol for the extraction, for it was a little difficult in the case of the samples of the minus sulphur stems to avoid the precipitation of a few crystals which seemed to be amide nitrogen. There was a little loss of this fraction in these samples, but not enough to affect the results materially. As shown by table II, the minus sulphur stems were very high in amide nitrogen as compared with the plus sulphur stems.

CARBOHYDRATE FRACTIONS.—The water extract for the sugar determinations was prepared as previously described (8). The sugars were determined by a combination of the Munson and Walker and Bertrand methods (18). Acid hydrolysis was used in the determination of total sugars. The solution was neutralized with 50 per cent sodium hydroxide, the exact end point being adjusted with 0.1 N acid and alkali. Phenolphthalein was the indicator used.

The residue was dried to constant weight, ground first in a coffee mill and then in a ball mill, so that it would pass through a 100 mesh sieve, and again dried to constant weight. Starch was determined in the powdered residue by salivary digestion by the method of NIGHTINGALE (21), the method being modified somewhat. The residue was boiled with water for only 3–5 minutes. It was then digested at 80° C. in an oven for 30 minutes. After salivary digestion, the extract was heated in a boiling water bath for 2.5 hours with 2.5 per cent HCl. After neutralization, the extract was cleared and deleaded before the reduction was made. Hemicelluloses were determined by the method of CLEMENTS (3), except that hydrochloric acid was used in place of sulphuric acid. The extract was cleared and deleaded as in the case of the starch extract. In both cases the Bertrand modification of the Munson and Walker method was used to determine the resulting sugar.

NITROGEN FRACTIONS.—Ammonia nitrogen, nitrate nitrogen, and amino nitrogen were determined by the methods of PHILLIPS (28). The reduced iron method of PUCHER, LEAVENWORTH, and VICKERY (31) was used for the determination of total nitrogen in the extract. The method was followed exactly in the reduction process. For the digestion process, copper sulphate was used as the catalyst instead of mercury, and potassium permanganate was not added. Sodium thiosulphate was not added before the distillation, of course. Total nitrogen in the residue was determined by the Gunning method (1). Total organic nitrogen was obtained by subtracting nitrate nitrogen from the total nitrogen; soluble organic nitrogen, by subtracting nitrate nitrogen from the total nitrogen of the alcoholic extract. Since 80 per cent alcohol was used for the extraction, this fraction is not the same as in the case of the water extraction method.

SULPHUR FRACTIONS.—An attempt was made to determine volatile sulphur in green, finely ground soy bean tissue by PETERSON'S

method (27) in order to determine whether there was danger of losing
any sulphur on drying the residue to constant weight. The method
did not prove to be very satisfactory, but the trials indicated the
presence of such an extremely small amount of volatile sulphur that
it was not considered worth while to determine this fraction. Total
sulphur and sulphate sulphur were therefore determined in the pow-
dered residue and in the extract after evaporating off the alcohol by
the official methods (1). For the sulphate determination on the resi-
due a water extract was made, and this extract as well as the alcohol-
free extract was freed of coagulable material by heating to boiling,
adding acetic acid to the hot solution, and filtering. Organic sul-
phate represents the difference between total sulphur and sulphate
sulphur.

MICROCHEMICAL METHODS.—Microchemical tests were made on
the green tissue for nitrates, reducing sugars, starch, and sulphates
by the methods of ECKERSON (10).

Data

EXTERNAL EFFECTS OF SULPHUR DEFICIENCY.—It took consider-
able time for the symptoms of sulphur deficiency to develop, but in
four weeks they were beginning to show, and in six weeks they were
pronounced. They were somewhat more marked in the plants grown
in 1933 than in those grown in 1934, but were similar in the two sets.
The main symptoms were the yellow-green color of the leaves, the
smaller leaflets, and the thinner stems of the sulphur deficient plants
as compared with the plus sulphur plants (fig. 1). Also the stems of
the former were harder than those of the latter. It was repeatedly
noticed that the upper leaves of the sulphur deficient plants (not
counting the small undeveloped leaves) became yellow first (fig. 2).
There was increasing greenness from the top to the bottom of the
plant. In certain cases the lower leaves also became yellow, but this
was attributed to the breakdown of the chlorophyll incident to the
death of the leaves. There was a tendency for the lower leaves, es-
pecially in the case of the minus sulphur plants, to die and fall off.
The difference in size of the leaves of the plus sulphur and minus
sulphur plants was pronounced. Some of the 1933 plants were al-
lowed to grow after the last sampling was made. On May 29, four
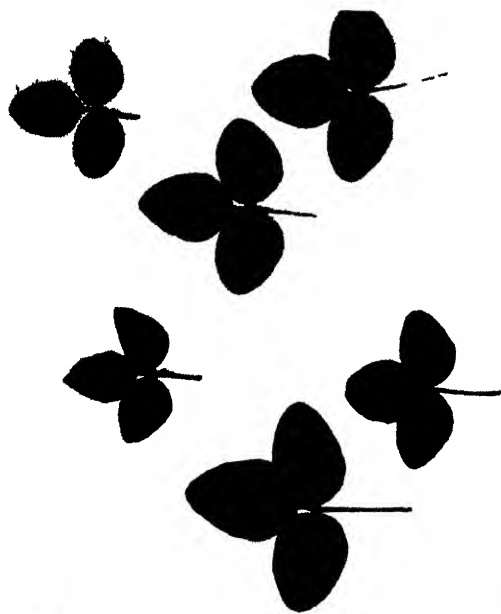
FIGS 1, 2 —Fig 1 (left), soy bean plants (May 5, 1933) minus sulphur plant on left, plus sulphur plant on right Fig 2 (right), soy bean leaves arranged according to position on plant (April 18, 1933) plus sulphur leaves on left; minus sulphur leaves on right Photographs by E W HOPKINS.

leaves from the tallest sulphur deficient plant and four leaves from corresponding levels on the tallest plus sulphur plant were blue printed and the area determined with a planimeter. The area of the minus sulphur leaves totaled 371.8 sq. cm.; that of the plus sulphur leaves, 786.3 sq. cm.

TABLE I

GREEN WEIGHT (100 PLANTS), PERCENTAGE MOISTURE, PERCENTAGE
DRY WEIGHT, TOP-ROOT RATIO

| | SAMPLES TAKEN | | | | | |
| | APRIL 22, 1933 | | MAY 5, 1933 | | APRIL 21, 1934 | |
	PLUS S	MINUS S	PLUS S	MINUS S	PLUS S	MINUS S
Green weight (gm.)						
Tops..................	557.2	439.4	1280.8	786.6	1364 9	873.9
Leaves................	239.5	180 2	533.3	326.0	507.4	351.4
Stems................	213.6	186.0	470.2	294.1	550.0	350.7
Roots................	231.1	214.0	580.0	516 3	506.9	500.1
Entire plant..........	788.3	653.4	1860.8	1302.9	1871.8	1374.0
Top-root ratio..........	2.411	2.052	2.208	1.523	2.730	1.747
Percentage moisture						
Leaves................	79.03	78.52	79.42	78.48	82.32	81.51
Stems................	81.05	78.49	81.08	79 99	83.44	83.05
Roots................	90.63	91.11	92.32	92.78	90.88	91.47
Percentage dry weight						
Leaves................	20.97	21.48	20.58	21.52	17.68	18.49
Stems................	18.95	21.51	18.20	22.10	16.56	16.95
Roots................	9.37	8.89	7.68	7.22	9.12	8.53

Stem elongation and the twining habit were prominent features of both series of plants. Of course, because of the use of electric lights the plants of neither series were normal (8, 29, 38). But omitting sulphur from the nutrient solution did not prevent stem elongation, although the minus sulphur plants were on the average not so tall as those grown with a complete nutrient solution (fig. 1). On April 19, 1934, when the plants were about seven weeks old, three of the tallest minus sulphur plants and three of the tallest plus sulphur plants were measured. The three minus sulphur plants measured 34, 34.5, and 34.75 inches in height; the three plus sulphur plants,

33.75, 36, and 37.75 inches. The stems of the sulphur deficient plants were decidedly smaller in diameter throughout their entire length than the stems of the plants grown with a complete nutrient solution, being in some cases less than 2 mm. in diameter at the tips.

Table I gives data for the green weight, percentage moisture, percentage dry weight, and top-root ratios of the plants. The top-root ratios are obtained by dividing the green weight of the tops per 100 plants by the green weight of the roots per 100 plants. They are somewhat smaller than they should be because of the impossibility of entirely removing the surface water of the roots. It is evident that the sulphur deficient plants were smaller and that this difference became greater the longer the plants grew. Omitting sulphur from the nutrient solution affected the tops more than it did the roots. The top-root ratios are consistently greater for the plus sulphur plants. The tops of the plus sulphur plants were more succulent, as is evidenced by the higher moisture content of the leaves and stems. This difference was in general greater for the 1933 plants than for those grown in 1934. It is reversed in the roots, but probably has little significance, since it is difficult to make an accurate determination of the moisture content of roots.

EFFECTS OF SULPHUR DEFICIENCY ON CHEMICAL COMPOSITION OF PLANT

NITROGEN FRACTIONS.—Data for the chemical composition of the soy bean plants are given in tables II–VII. Only in the case of the 1934 plants are data recorded for the entire plant. The stem tips, petioles, etc., of the plants grown in 1933 were not analyzed. The percentages are figured on a dry weight basis. Since the plus sulphur plants have a lower percentage dry weight, this method of recording the data favors these plants in comparison with the minus sulphur plants; and in one or two instances reverses the difference which would exist if the data were calculated on a green weight basis. But percentage composition of roots figured on a green weight basis means little. In order, therefore, to have a uniform plan of recording the data for all parts of the plant and to save space, the data are recorded for the dry weight basis only.

Table II records data for the nitrogen fractions on a percentage

TABLE II

NITROGEN FRACTIONS

(PERCENTAGE, DRY WEIGHT BASIS)

	SAMPLES TAKEN					
	APRIL 22, 1933		MAY 5, 1933		APRIL 21, 1934	
	PLUS S	MINUS S	PLUS S	MINUS S	PLUS S	MINUS S
Total N						
Leaves	4 219	3 627	4 271	4 019	6 072	6 165
Stems	2 013	2 421	1 408	2 928	1 172	2 885
Roots	2 926	2 889	2 445	2 698	2 737	2 406
Stem tips, etc					2 622	3 165
Total organic N						
Leaves	4 127	3 435	4 218	3 874	5 999	5 934
Stems	1 705	2 154	1 226	2 736	1 058	2 658
Roots	2 448	2 474	2 126	2 195	2 598	2 160
Stem tips, etc					2 420	2 949
Soluble organic N						
Leaves	0 366	0 525	0 428	0 585	0 342	0 345
Stems	0 500	1 084	0 259	1 273	0 143	1 252
Roots	0 120	0 528	0 151	0 796	0 170	0 132
Stem tips, etc					0 249	0 770
Ammonia N						
Leaves	0 005	0 010	0 011	0 013	0 007	0 005
Stems	0 009	0 018	0 007	0 024	0 006	0 016
Roots	0 016	0 020	0 018	0 034	0 015	0 016
Stem tips, etc					0 010	0 015
Amino N						
Leaves	0 147	0 301	0 174	0 200	0 200	0 186
Stems	0 234	0 498	0 205	0 536	0 169	0 544
Roots	0 158	0 308	0 174	0 351	0 142	0 155
Stem tips, etc					0 218	0 254
Amide N						
Leaves	0 028	0 088	0 049	0 085	0 061	0 060
Stems	0 141	0 317	0 060	0 322	0 025	0 372
Roots	0 038	0 160	0 053	0 187	0 057	0 085
Stem tips, etc					0 049	0 092
Nitrate N						
Leaves	0 092	0 192	0 053	0 145	0 073	0 231
Stems	0 308	0 267	0 182	0 192	0 114	0 227
Roots	0 478	0 425	0 329	0 503	0 139	0 246
Stem tips, etc					0 202	0 216

basis. There is an accumulation of ammonia nitrogen, amino nitro-
gen, amide nitrogen, and nitrate nitrogen in the minus sulphur as
compared with the plus sulphur plants. The stems show this accu-
mulation more than the roots, except in the case of nitrate nitrogen.
The difference becomes greater the longer the plants grow. Owing
largely to this great accumulation of the soluble organic nitrogen
fractions, the percentage of soluble organic nitrogen, total organic
nitrogen, and even total nitrogen is greater in the stems of the sul-
phur deficient plants than in those of the plus sulphur plants. This
is not consistently true of the leaves and roots.

It should be mentioned that in certain cases the sum of the solu-
ble nitrogen fractions, including the nitrates, is a little greater than
the total nitrogen in the alcoholic extract (soluble organic nitrogen
plus nitrates). This indicates some slight error in the analyses of
these samples. WEBSTER (36) noted a decrease in amino nitrogen
and an increase in ammonia nitrogen in alcoholic solution. STUART
(35) reports difficulties in the determination by the Van Slyke meth-
od of amino nitrogen in the alcoholic extract of apple tissue, owing to
the presence of some gas not absorbed by the potassium perman-
ganate. But there was no evidence of a discrepancy such as WEBSTER
reports. Ammonia nitrogen was always low in amount, and no diffi-
culty such as STUART reports was experienced in the determination
of amino nitrogen.

The facts that the discrepancy just mentioned occurs only in part
of the samples, that when it occurs it is nearly always small, and
that the analyses of plants at different stages of growth and of
plants grown in different years give similar results, certainly indicate
that the data of table II give rather closely the correct situation as to
the percentage and distribution of the nitrogen fractions in the plus
and minus plants.

Table III records the data for the nitrogen fractions on an abso-
lute basis. Despite the greater growth of the plus sulphur plants, the
totals for the sulphur deficient plants are greater for soluble organic
nitrogen, ammonia nitrogen, amino nitrogen, amide nitrogen, and
nitrate nitrogen than for the plus sulphur plants, except in the case
of the ammonia determination of April 21, 1934, and the nitrate
total of April 22, 1933. There is some variation in regard to these

TABLE III

NITROGEN FRACTIONS

(ABSOLUTE GRAMS PER 100 PLANTS)

	SAMPLES TAKEN					
	APRIL 22, 1933		MAY 5, 1933		APRIL 21, 1934	
	PLUS S	MINUS S	PLUS S	MINUS S	PLUS S	MINUS S
Total N						
Leaves..........	2.118	1.404	4.688	2.820	5.448	4.006
Stems..........	0.815	0.968	1.204	1.895	1.067	1.715
Roots..........	0.633	0.551	1.093	1.005	1.265	1.026
Stem tips, etc.....	....	....	....	....	0.889	0.738
Total.........	3.566	2.923	6.985	5.720	8.669	7.485
Total organic N						
Leaves..........	2.072	1.330	4.630	2.718	5.383	3.856
Stems..........	0.691	0.861	1.049	1.771	0.963	1.580
Roots..........	0.530	0.047	0.947	0.818	1.201	0.921
Stem tips, etc.....	....	....	....	....	0.821	0.695
Total.........	3.293	2.238	6.626	5.307	8.368	7.052
Soluble organic N						
Leaves..........	0.184	0.203	0.470	0.410	0.307	0.224
Stems..........	0.203	0.433	0.222	0.824	0.130	0.744
Roots..........	0.026	0.101	0.068	0.297	0.078	0.056
Stem tips, etc.....	....	....	....	....	0.085	0.154
Total.........	0.413	0.737	0.760	1.528	0.600	1.178
Ammonia N						
Leaves..........	0.002	0.004	0.013	0.009	0.007	0.003
Stems..........	0.003	0.007	0.006	0.015	0.005	0.009
Roots..........	0.003	0.003	0.008	0.013	0.007	0.007
Stem tips, etc.....	....	....	....	....	0.004	0.003
Total.........	0.008	0.014	0.027	0.037	0.023	0.022
Amino N						
Leaves..........	0.073	0.116	0.191	0.140	0.180	0.120
Stems..........	0.094	0.199	0.176	0.347	0.154	0.323
Roots..........	0.034	0.058	0.077	0.131	0.065	0.066
Stem tips, etc.....	....	....	....	....	0.073	0.050
Total.........	0.201	0.373	0.444	0.618	0.472	0.559
Amide N						
Leaves..........	0.014	0.034	0.054	0.060	0.054	0.039
Stems..........	0.057	0.127	0.052	0.208	0.022	0.221
Roots..........	0.008	0.030	0.024	0.069	0.026	0.036
Stem tips, etc.....	....	....	....	....	0.016	0.018
Total.........	0.079	0.191	0.130	0.337	0.118	0.314
Nitrate N						
Leaves..........	0.046	0.074	0.058	0.102	0.065	0.150
Stems..........	0.124	0.107	0.155	0.124	0.104	0.135
Roots..........	0.103	0.080	0.146	0.187	0.064	0.105
Stem tips, etc.....	....	....	....	....	0.068	0.043
Total.........	0.273	0.261	0.359	0.413	0.301	0.433

fractions in different parts of the plant. As already stated, data were secured for the entire plant only for the analysis of April 22, 1934. But the totals for total organic nitrogen and total nitrogen are consistently greater for the plants grown in a complete nutrient solution, showing, as is to be expected, that the large plus sulphur plants synthesized a larger amount of nitrogenous material. Even here, however, the stems of the minus sulphur plants are higher, owing to their greater accumulation of the soluble organic nitrogen compounds.

Table IV records the data for the carbohydrate fractions on a percentage basis. The main points are that in general the sugars are higher in the plants grown in a complete nutrient solution but that starch and hemicellulose accumulate in the sulphur deficient plants. This difference in starch is greater in the stems and roots of the May 5, 1933, analysis than at the time of the earlier analysis, although the actual percentages are less in all parts of the plant except in the plus sulphur roots. Although the actual starch content of the plant is less later in the growth period, the hemicellulose percentage is greater, especially in minus sulphur leaves, and both the plus and minus sulphur stems. This may mean that as the plants get older the hemicellulose increases at the expense of the starch. Sulphur deficiency does not result in as great a piling up of hemicellulose as of starch; yet the minus sulphur leaves of the May 5, 1933, analysis have more than 2 per cent more hemicellulose than the plus sulphur leaves. The difference in favor of the high sulphur stems of the May 5 analysis is reversed if the percentages are calculated on the green weight basis. Adding the total sugars and starch together, it is found that in general the sulphur deficient plants of the two 1933 analyses have a higher percentage, but this is not true of the 1934 plants. When the percentage of total carbohydrates is figured, however, the low sulphur plants of all three analyses are higher, in most of their parts, although the differences are small in the 1934 plants. Total sugars plus starch and total carbohydrates were figured for the total weight of leaves, stems, and roots of the two 1933 samplings and for the entire plant of the 1934 series, with the results given in the last two horizontal columns of table V. It may be noted that with the carbohydrates figured in either one of these ways, the 1933

sulphur deficient plants of both analyses were high carbohydrate plants as compared with the plus sulphur plants; while in the case

TABLE IV

CARBOHYDRATE FRACTIONS

(PERCENTAGE, DRY WEIGHT)

	SAMPLES TAKEN					
	APRIL 22, 1933		MAY 5, 1933		APRIL 21, 1934	
	PLUS S	MINUS S	PLUS S	MINUS S	PLUS S	MINUS S
Total sugars						
Leaves .	3 792	4 479	3 091	2 676	1 688	1 544
Stems	3 218	1 580	2 692	1 070	2 3084	0 862
Roots	3 084	2 588	3 412	2 461	1 822	1 315
Stem tips, etc					1 603	0 926
Reducing sugars						
Leaves	1 245	1 174	1 109	0 930	0 682	0 481
Stems	1 411	0 411	1 653	0 300	1 794	0 404
Roots	1 461	0 872	1 562	1 048	1 263	0 712
Stem tips, etc					1 416	0 559
Sucrose						
Leaves	2 419	3 139	1 883	1 659	0 955	1 012
Stems	1 717	1 110	0 986	0 732	0 487	0 440
Roots	1 343	1 630	1 757	1 341	0 530	0 572
Stem tips, etc					0 179	0 349
Starch						
Leaves	10 564	14 140	7 560	7 067	2 324	2 338
Stems	8 586	10 677	3 894	8 386	1 896	2 318
Roots	2 740	2 614	2 071	3 334	1 835	1 811
Stem tips, etc					2 875	3 324
Hemicelluloses						
Leaves	4 784	5 227	4 706	7 047	4 790	5 046
Stems	9 686	10 173	11 878	11 767	12 073	11 612
Roots	9 480	9 228	8 644	8 888	7 578	8 718
Stem tips, etc	•				8 259	8 536
Total sugars plus starch						
Leaves	14 356	18 619	10 651	9 743	4 012	3 882
Stems	11 804	12 257	6 586	9 457	4 204	3 180
Roots	5 824	5 202	5 483	6 586	3 657	3 126
Stem tips, etc.					4 478	4 250
Total carbohydrates •						
Leaves	19 140	23 846	15 357	16 890	8 802	8 928
Stems	21 490	22 430	18 464	21 224	16 277	14 792
Roots	15 304	14 430	14 127	15 474	11 235	11.844
Stem tips, etc. .	. .				12 737	12 786
Total sugars plus starch ..	11 976	13 402	8 240	8 780	4 076	3 505
Total carbohydrates	18 651	21 431	16 236	18 000	12 354	11 869

of the 1934 plants the difference is slightly in favor of plants grown in a complete nutrient solution.

In table V the carbohydrates are figured on an absolute basis. The totals for the sugars are higher for the plus sulphur plants, and in general this is true for the different parts of the plant. This is to be expected, since the high sulphur plants were larger and also had a larger percentage sugar content. Despite the higher percentage of starch and hemicellulose of the sulphur deficient plants, however, due to the larger amount of tissue formed by the plus sulphur plants, the totals for these substances are larger for the latter plants except in the case of starch of the April 22, 1933, analysis. This is not consistently true, however, of the different parts of the plant, except for starch of the April 21, 1934, analysis. The totals for total sugars plus starch and total carbohydrates are consistently larger for the high sulphur plants.

SULPHUR FRACTIONS.— Tables VI and VII record a few results for the sulphur fractions of the 1934 plants. The plants grown in a complete nutrient solution have a higher percentage of total sulphur and sulphate sulphur; but a greater proportion of the total sulphur of the sulphur deficient plants is present in the form of organic sulphur than is true of the plus sulphur plants. The minus sulphur leaves have a higher percentage of organic sulphur than the plus sulphur, while the stems of the two series are the same in this respect. There was very little organic sulphur or sulphate sulphur in the alcoholic extract. Nearly all of both fractions was found in the residue. On an absolute basis, the plants grown in a complete nutrient solution are higher in all three fractions.

MICROCHEMICAL DATA.—Microchemical tests were made for starch, reducing sugars, sulphates, and nitrates. The data do not add much to the macrochemical data recorded in the tables, but in general confirm them. One point of interest appeared in the microchemical tests for starch on the 1934 plants. The quantitative data show that there is a higher percentage of starch in the minus sulphur stems, although the difference is small. Microchemical tests indicated that near the base the plus sulphur stems were somewhat higher in starch but that near the tip the two series were about equal. In fact, tests on some of the plants indicated that the sulphur deficient

TABLE V

CARBOHYDRATE FRACTIONS

(ABSOLUTE GRAMS PER 100 PLANTS)

	SAMPLES TAKEN					
	APRIL 22, 1933		MAY 5, 1933		APRIL 21, 1934	
	PLUS S	MINUS S	PLUS S	MINUS S	PLUS S	MINUS S
Total sugars						
Leaves..........	1.904	1.733	3.393	1.878	1.514	1.003
Stems..........	1.303	0.632	2.303	0 693	2.102	0.513
Roots..........	0.667	0.492	1.520	0.917	0.842	0.561
Stem tips, etc.....					0.549	0.185
Total........	3.207	2.857	7 216	3.488	5.007	2.262
Reducing sugars						
Leaves..........	0 625	0 454	1.217	0 652	0.611	0.312
Stems..........	0.571	0.164	1.415	0.194	1.634	0.240
Roots..........	0.316	0.165	0.695	0.390	0.584	0.304
Stem tips, etc.....					0.480	0.111
Total........	1.512	0.783	3.327	1.236	3.309	0.967
Sucrose						
Leaves..........	1.214	1 215	2.066	1.164	0.857	0.657
Stems..........	0.695	0.444	0.844	0.474	0 444	0.261
Roots..........	0.290	0 310	0.782	0.500	0.245	0 244
Stem tips, etc.....					0.060	0.069
Total........	2.199	1.969	3.692	2.138	1.606	1.231
Starch						
Leaves..........	5.305	5.474	8.298	4.959	2 085	1.519
Stems..........	3.476	4.270	3.332	5.429	1.726	1.378
Roots..........	0.593	0.497	0.922	1.242	0.848	0.772
Stem tips, etc.....					0 975	0.665
Total........	9 374	10.241	12.552	11.630	5.634	4.334
Hemicelluloses						
Leaves..........	2.402	2.023	5.166	4 945	4.298	3.279
Stems..........	3.921	4 069	10.164	7.617	10.996	6.903
Roots..........	2.052	1.755	3.850	3.311	3.503	3.719
Stem tips, etc.....					2.801	1.709
Total........	8.375	7.847	19.180	15.873	21.598	15.610
Total sugars plus starch						
Leaves..........	7.209	7.207	11.691	6.837	3.599	2.522
Stems..........	4.779	4.902	5.635	6.122	3.828	1.891
Roots..........	1.260	0.989	2.442	2.159	1.690	1.333
Stem tips, etc.....					1.524	0.850
Total........	13.248	13.098	19.768	15.118	10.641	6.596
Total carbohydrates						
Leaves..........	9.611	9.230	16.857	11.782	7.897	5.801
Stems..........	8.700	8.971	15.799	13.739	14.824	8.794
Roots..........	3.312	2.744	6.292	5.470	5.193	5.052
Stem tips, etc.....					4.328	2.559
Total........	21.623	20.945	38.948	30.991	32.242	22.206

stems had a little higher percentage of starch near the tip than the plants grown in a complete nutrient solution. Since, when the entire stem is analyzed together the low sulphur stems have a higher percentage, it seems likely that some of the starch present in the lower part of sulphur deficient stems is hydrolyzed to sugar, and that the sugar is translocated to the tips of the plants, where part of it is changed back to starch. It is probable that, accompanying this

TABLE VI

SULPHUR FRACTIONS

(PERCENTAGE, DRY WEIGHT)

	TOTAL S		SULPHATE S		ORGANIC S	
	LEAVES	STEMS	LEAVES	STEMS	LEAVES	STEMS
Plus S	0.430	0.270	0.223	0 223	0.207	0.047
Minus S...........	0.342	0.123	0.106	0.076	0.236	0.047

TABLE VII

SULPHUR FRACTIONS

(ABSOLUTE GRAMS PER 100 PLANTS)

	TOTAL S		SULPHATE S		ORGANIC S	
	LEAVES	STEMS	LEAVES	STEMS	LEAVES	STEMS
Plus S.....	0.386	0.246	0.200	0.203	0.186	0.043
Minus S...........	0 222	0.073	0.069	0.045	0.153	0.028

starch transfer, some of the chloroplasts in the lower part of the minus sulphur stems break down, the proteins being hydrolyzed and the material translocated to the tips of the plants, where it is used in stem elongation, as will be discussed later.

BORON INJURY.—The question naturally arises whether boron injury may not in part account for the differences between the plus sulphur and minus sulphur plants of 1933. While it is possible that this may be true, it is not thought that the results obtained are much affected by this factor. In the first place, although exact counts were not made, boron deficiency seemed to affect the plants of the two

series about equally. Again, although the April 22, 1933, analysis was made before there was much recovery from boron injury and included some plants with dead tips, at the time of the May 5, 1933, analysis most of the plants had recovered and had put out long side shoots. All the plants sampled at this time had well developed side shoots. Yet the two analyses show similar differences between the plus and minus sulphur plants except that they are often greater in the case of the latter analysis, as would be expected. Finally, no boron injury developed in the 1934 plants; yet the differences in the chemical composition of the two series are similar to those of the 1933 plants, although decidedly smaller in the case of starch and hemicellulose.

SULPHUR DEFICIENCY IN OTHER PLANTS.—Several other plants were grown in 1934 to compare with the soy bean. These were sunflower, rape, kale, and mustard. The symptoms of sulphur deficiency were about the same as in the soy bean. The minus sulphur plants of each series were smaller, shorter, and not so green as the plus sulphur plants. The effects of sulphur deficiency on stems and leaves were very pronounced in the sunflower, the leaves being smaller and the stems thinner. While the mustard plainly showed the effects of lack of sulphur, it was more in the size of the plants than in color. This is perhaps not strange, since so much of the sulphur is present in mustard oil rather than in protein. The higher mustard oil content of the plus sulphur mustard leaves was very evident to the taste, which was much more acrid than in the case of the minus sulphur leaves. This difference was not so marked in the case of the other Cruciferae, kale, and rape.

No analyses were made of any of these plants. Table VIII gives the green weights and the top-root ratios. The top-root ratios are figured on the green weight basis and are smaller than they should be because of the excess surface water on the roots. It was impossible to remove all of this with filter paper. No analyses were made. It is planned later to grow these plants in quantity and make analyses to see how they compare in chemical composition with the soy bean.

Discussion

COLOR, SIZE OF LEAVES, THICKNESS OF STEMS.—The main symptoms of sulphur deficiency in the soy bean were the yellow-green

color of the leaves, the smaller size of the leaves, and the thinner stems as compared with the plants grown in a complete nutrient solution.

The effects of sulphur deficiency on the size and color of the plant have been known for a long time. Even during the early history of our government sulphur in the form of gypsum was in common use, and resulted in increased yield. The darker green color of the plants was also noticed. CROCKER (4) reviews some of this early literature. More recently in the northwest the effects of sulphur fertilizers in producing larger, darker green plants has been noted in the case of alfalfa (26, 32). Great increases in yield have been obtained and it

TABLE VIII

GREEN WEIGHT PER 25 PLANTS, TOP-ROOT RATIO

	SUNFLOWER		KALE		RAPE		WHITE MUSTARD	
	HIGH S	LOW S	HIGH S	LOW S	HIGH S	LOW S	HIGH S	LOW S
Tops	780 0	430 2	201 6	43 4	351 2	223 8	155 4	104 3
Roots	130 0	109 3	35 4	8 2	52 9	38 0	54 4	47 6
Entire plant	910 0	539 5	237 0	51 6	404 1	311 8	209 8	151 9
Top root ratio	6 000	3 933	5 690	5 260	6 631	5 874	2 856	2 188

has been definitely proved that the effect is due to the sulphur component of the fertilizer used.

Until rather recently most of the work with sulphur fertilizers has been done in the field, or in the greenhouse with soil cultures. Plants belonging to either the Leguminosae or the Cruciferae have given the best response. This is naturally true since in general the plants of these two families are highest in sulphur owing to the high content of the legumes in protein which contains sulphur and of the crucifers in mustard oils and other sulphur containing compounds. But using sand cultures, DULEY (6) noted the effects of sulphur deficiency in producing smaller, less green corn plants. And recently NIGHTIN-GALE (22) in the tomato, and STOREY and LEACH (34) in the tea bush secured symptoms of sulphur deficiency that correspond closely to those obtained in the soy bean.

The gradation of greenness in regard to position of the leaves on

the plant seems to vary with different plants. While it was not per-
haps absolutely regular, the upper leaves of the soy bean generally
became yellow first. There was an increase in greenness from the top
to the bottom of the plant (fig. 2). Sometimes the lower leaves also
became yellow, but since many of the lower leaves died and fell off,
the yellow color was probably due to the decomposition of the chlor-
ophyll which accompanied the general breakdown of the cell con-
tents as death approached. This gradation of greenness due to sul-
phur deficiency seems to correspond with the results of STOREY and
LEACH (34) for the tea bush, but is in contrast to the results of
NIGHTINGALE (22) for the tomato. In the case of the tomato the
lower leaves became yellow first.

As NIGHTINGALE points out (22), the symptoms of sulphur de-
ficiency are similar to the deficiency of other elements, although the
gradation of greenness varies. A nitrogen deficient plant is yellow
and has small leaves and stems (15). And at certain stages, at least,
the symptoms of lack of potassium (24), phosphorus (12), or calcium
(25) are similar to those of sulphur. That the effects of a deficiency
of each of these elements should be similar, especially in regard to
color, is perhaps not strange, for ECKERSON (11) has shown that
when these elements are lacking in the nutrient solution the tissues
of the resulting plants have a low reducase activity. So the use of
nitrates in the synthetic processes of the plant is interfered with and
nitrogen is necessary for the synthesis of chlorophyll and the proteins
of the chloroplast. In the case of calcium there is also poor absorp-
tion of nitrates (25). Thus it would seem that these elements are
having their effects indirectly by interfering with the assimilation of
nitrates. NIGHTINGALE (22) states: "The minus-sulphur [tomato]
plants looked as if they had been gradually but not completely
limited as to their nitrogen supply." This was true also of the soy
bean. While the plants were chlorotic, this condition was not so
pronounced as in advanced stages of nitrogen deficiency. And ECK-
ERSON (11) found that although sulphur deficiency caused a lower
amount of reducase in the tomato, there was still enough present
to allow the reduction of nitrates to go on slowly. Sulphur deficiency
does not cause as great a reduction in reducase as deficiency of po-
tassium, phosphorus, or calcium.

Although the chlorotic condition of minus sulphur plants would

seem to be due mainly to poor nitrate assimilation, two other points should be mentioned. One is that although sulphur is not needed as a building stone for the synthesis of chlorophyll, it is needed for the synthesis of most if not all plant proteins. So it is probably needed for the synthesis of the proteins of the chloroplast and its absence might result in the development of fewer chloroplasts, and thus affect the degree of greenness to a certain extent. Also, the synthesis of chlorophyll is one of the life processes of the plant, and the deficiency of any element necessary for the healthy state and proper functioning of the plant may no doubt affect chlorophyll development.

STEM ELONGATION.—NIGHTINGALE (22) found that the minus sulphur tomato plants were as tall as, and in some cases taller than, the plants grown in a complete nutrient solution, but they were much smaller in diameter. Much the same situation is found in the soy bean. The stems of both plus and minus sulphur plants elongated remarkably and assumed the twining habit (fig. 1). Of course the habit of neither set of plants was normal, because of the use of electric lights. Stem elongation has often been noticed, when electric lights have been used or when certain parts of the spectrum have been eliminated (8, 29, 38). But the point is that leaving sulphur out of the nutrient solution did not prevent stem elongation. The sulphur deficient plants, although not so tall on the average as the plants grown in a complete nutrient solution, still showed decided stem elongation. Some of them were about as tall as the plus sulphur plants, but were very small in diameter, in some cases being less than 2 mm. in diameter at the tips.

This is a rather unique situation. Deficiency of an essential element usually causes the stunting of the plant, which is shown not only in smaller plant parts but in shorter stems. Deficiency of sulphur results in less production of tissue, the plant parts are smaller (table I), but the stem may be as long as those of plants grown in a complete nutrient solution. Only in special situations does this seem to be true of other elements, as, for example, when plants deficient in nitrogen (23), potassium (24), or calcium (25) are placed in darkness. Then there is marked stem elongation. This subject will be referred to again in connection with a discussion of the effects of sulphur deficiency on the organic nitrogen fractions.

ROOT DEVELOPMENT.—Minus sulphur soy bean plants had poor root development, but the roots were not stunted as much as the tops, as shown by the top-root ratios (table I); in fact in the 1934 plants the roots of the sulphur deficient plants weighed almost as much as the plants grown in a complete nutrient solution.

This effect of sulphur deficiency in causing poor root development has been known for a considerable time, although the data given do not always permit a comparison of the effects on tops and roots. REIMER and TARTAR (32) emphasize the increased root development of alfalfa due to sulphur fertilization. Recently NIGHTINGALE (22) found that the roots of sulphur deficient tomato plants were of small diameter because there was not much cambial development and therefore very little secondary thickening. STOREY and LEACH (34) state that the roots of the tea bush suffering from yellows (which they show is a sulphur deficiency disease) may be poorly developed.

It is interesting to compare the effects of sulphur deficiency on root development with the effects of the deficiency of other elements. NIGHTINGALE (22) states that the roots of minus sulphur plants are similar to the roots of low nitrogen plants, and he has shown (23) that plus nitrate solutions cause greater growth of tops than of roots; or to state it conversely, nitrate deficiency stunts the tops more than it does the roots. This is similar to the effects of sulphur deficiency on the soy bean. HARRISON (13) found that nitrates increased the growth of the tops of golf grasses but not of the roots. WELTON (37) found that the roots of soy bean weighed 6.2 per cent as much as the tops when the plants were grown in sand, but only 3.4 per cent as much when grown in manure. RUSSELL (33) emphasizes the influence of phosphorus on root development. He states that the root systems of phosphate deficient cereals are stunted and more so than the tops, and that the addition of phosphates to such plants causes greater increase in tops than roots. But superphosphate was apparently used in these experiments, and as CROCKER (5) points out, the effect may have been due partly to the sulphates present. ECKERSON (12) studied the effects of phosphate deficiency without this complication. Her pictures show a decided stunting of both roots and tops. No weights are given.

EFFECTS OF SULPHUR DEFICIENCY ON CHEMICAL
COMPOSITION OF PLANT

ACCUMULATION OF CARBOHYDRATES AND NITRATES.—Nitrates and carbohydrates may accumulate in plants as a result of several conditions. Nitrogen starved plants are high in carbohydrates (**15, 23**). This condition can be brought about either by withholding nitrates from the nutrient solution, in which case of course nitrates will be low or absent in the plant, or with nitrates in the nutrient solution, subjecting the plant to some condition which restricts nitrate reduction and thus the synthesis of organic nitrogenous compounds for which both nitrates and carbohydrates are necessary. In the latter case both nitrates and carbohydrates accumulate. ECKERSON (**11, 12**) has shown that tomato plants deficient in potassium or phosphorus are low in reducase. She found that the minus phosphorus tomato plants were high in carbohydrates and nitrates, and NIGHTINGALE (**24**) has shown that the same thing may be true of potassium deficient tomato plants. Carbohydrates accumulate in calcium deficient tomato plants (**25**), but here, although the reducase content is low, absorption of nitrates is poor and this alone would no doubt account for the accumulation of carbohydrates. Because of poor absorption, nitrates are not high in amount.

ECKERSON (**11**) found that sulphur deficient tomato plants are also low in reducase. NIGHTINGALE (**22**) found that nitrates and carbohydrates accumulate in these plants, and he states that this is due to the poor reduction of nitrates and synthesis of amino acids and various other organic nitrogenous compounds. A similar situation exists in the soy bean and it is no doubt to be explained on the same basis. The minus sulphur soy bean plants are high in nitrates. But in regard to the carbohydrates the situation is not so clear as in the tomato. Reducing sugars, sucrose, and starch accumulate in the sulphur deficient tomato plants. Hemicelluloses were not determined. Starch and hemicellulose are high in the minus sulphur soy bean plants, but the sugars are rather uniformly higher in the plus sulphur plants (table IV). Also, when total sugars plus starch or total carbohydrates are figured for the entire plant, the 1933 plants are high carbohydrate plants, and in general the same thing is true for the different parts of the plant. So the preceding explanation of

carbohydrate accumulation might be regarded as true for these plants. But when the carbohydrates are figured in these ways for the entire plant of the 1934 experiment, the plus sulphur plants are somewhat higher. And in the case of the different parts of the plant of the 1934 series, although total carbohydrates are a little higher in most cases in the minus sulphur plants, the reverse is true of total sugars plus starch. These plants, therefore, do not show the accumulation of carbohydrates as much as those of 1933. For the reasons just outlined, the effect of sulphur deficiency on carbohydrate accumulation is not so clear in the soy bean as in the tomato. But it would seem that the deficiency of this element may in some plants cause the accumulation of certain forms of carbohydrates rather than of all carbohydrates, for the plants of both years are uniform in regard to the higher percentages of starch and hemicellulose of the minus sulphur plants.

Table IV shows that the differences between the sulphur deficient plants and those grown in a complete nutrient solution are smaller for starch and hemicellulose for the 1934 plants than for those grown in 1933. It may be that variations in temperature and light conditions of the two years account for this, and of course these factors may also be of importance in accounting for the differences noted for relative accumulation of carbohydrates in the plants of the two years when figured as total sugars plus starch or total carbohydrates. For the reasons already noted, it is not thought that the boron deficiency which developed in the 1933 plants accounts for the differences.

It is not known in what degree hemicelluloses serve as reserve food materials of the plant. NIGHTINGALE (21) considers that hemicelluloses are not so important in the plant's metabolism as are starch and dextrin. CLEMENTS (3), however, considers that the hemicelluloses may be rather important metabolically. There is perhaps a little support in the data of table IV for the idea that the hemicelluloses may serve as temporary food reserves and therefore be important in the metabolism of the plant. At least the differences between the plus and minus plants in hemicellulose content is similar to, although not so great as (especially in the 1933 plants), the differences in starch.

ORGANIC NITROGEN FRACTIONS.—NIGHTINGALE (22) finds that

minus sulphur tomato plants are high in the soluble organic nitrogenous fractions. This is also true of the soy bean, especially in the stems (table II). This situation has been found to occur in plants as a result of various conditions. For example, a plant that is actively vegetative owing to an abundance of nitrates is high in these organic forms of soluble nitrogen, as compared with the nitrogen deficient plant (23). Here the organic nitrogenous compounds are being synthesized more rapidly than they can be combined to form protein. If plants deficient respectively in nitrogen (23) or potassium (24) are put in continuous darkness, there is stem elongation; and accompanying this, amide nitrogen, amino nitrogen, etc., increase in percentage and carbohydrates decrease. Here proteolysis occurs. Proteins are being hydrolyzed and reutilized in stem elongation. Sometimes the deficiency of an essential element results in the piling up of these soluble organic nitrogenous compounds even when the plants are in the light. NIGHTINGALE (24) found this true of potassium deficient tomato plants at a late stage, and it has also been found to be true of phosphorus deficient tomato plants (16, 17). NIGHTINGALE (22) attributes this situation in minus potassium or minus phosphorus tomato plants to proteolysis, which occurs shortly before the death of the plant and is accompanied by rather rapid stem elongation.

Sulphur deficient tomato plants are low in reducase (11). This no doubt is also true of the soy bean. Therefore the reduction of nitrates and the synthesis of proteins is much restricted. So the accumulation of these organic forms of soluble nitrogen could not be due to their rapid synthesis such as occurs in a highly vegetative plant supplied with an abundance of nitrates. The accumulation occurred while the plants were exposed to the full light period; it was not a case of proteolysis in darkness. There was no evidence that the minus sulphur plants were about to die. In fact, some of the sulphur deficient plants of 1933 were allowed to grow more than two weeks after the last samples were taken and they were still growing vigorously when the experiment was discontinued. Proteolysis which sometimes occurs shortly before the death of the plant does not seem to account for the piling up of these compounds. It seems to be due in the soy bean, as in the tomato (22), to a special type of proteolysis.

Both kinds of plants are able, under normal light conditions and while they are still healthy, to break down proteins, translocate the nitrogenous material, and reutilize it in stem elongation and leaf development. It is this power of the plants which accounts for the stem elongation already mentioned.

As has been pointed out in other cases of proteolysis (22), there is a decrease in the percentage of carbohydrates. But the sulphur deficient tomato plants were at all times high in reducing sugars, sucrose, and starch; yet they were actively breaking down proteins while these carbohydrates were accumulating. It is this fact which makes this a special case of proteolysis. The carbohydrate accumulation situation in the soy bean, however, seems to be somewhat different from that in the tomato. While the minus sulphur soy bean plants were higher in starch and hemicelluloses than the plus sulphur plants, they were decidedly lower in sugars, and the difference became greater in the later stages (table IV), indicating that the more directly available carbohydrates are lower in the sulphur deficient plants which are carrying on proteolysis. Also, although total sugars plus starch and total carbohydrates figured for the entire plant are higher in the minus sulphur plants of 1933, the reverse is true of the 1934 plants. So the carbohydrate situation in the soy bean might perhaps be considered to be more in line with that usually accompanying proteolysis than is true of the tomato.

There must have been, however, considerable synthesis of nitrogenous compounds by the minus sulphur plants, especially during the early part of the growth period, before reducase became deficient. A consideration of the data on an absolute basis becomes interesting in this connection (table III). The data are figured as grams per 100 plants. On April 22, 1933, the leaves, stems, and roots of the plus sulphur plants contained 32.93 mg. per plant of total organic nitrogen; the minus sulphur plants, 22.38 mg. This had increased by May 5 to 66.26 and 53.07 respectively. It is unfortunate that the stem tip, petioles, etc., were not analyzed. This was done in 1934, giving a total for the plus sulphur plant of 83.68 mg. per plant and the minus sulphur plant of 70.52 mg. The soy beans weighed on the average 167 mg. each. The seeds were not analyzed, but HENRY and MORRISON (14), in their compilation of the analyses of feeding ma-

terials, give 36.5 per cent as the average crude protein content of soy bean seeds (average of 121 analyses). Each seed then contains 60.9 mg. of crude protein, or, dividing by the usual factor, 6.25, 9.7 mg. of N. Making due allowance for differences in the protein content of different varieties and samples of soy bean seed, and regarding all of this protein as being hydrolyzed and translocated to the developing plant (which of course is no doubt untrue), it is seen that there must have been considerable synthesis of nitrogenous material by the sulphur deficient plants, and that this was going on more or less actively late in the growth period (compare data for April 22 and May 5). As already mentioned, ECKERSON found that sulphur deficiency does not result in as great a reduction of reducase in the tomato as the deficiency of other elements (11). There is enough reducase present to allow reduction of nitrates to go on slowly. With sulphates omitted from the nutrient solution, amino acids could not be combined to form proteins, since most, probably all, plant proteins contain sulphur. Therefore this newly synthesized nitrogenous material would accumulate in various soluble forms of nitrogen, and might account in part at least for the accumulation of the soluble forms of nitrogen in the minus sulphur plants (table II). With no sulphates in the nutrient solution it would not be expected that there would be present much cysteine, glutothione, or other sulphur containing nitrogenous compounds; and NIGHTINGALE (22) found these compounds largely absent from the organic sulphur of sulphur deficient tomato plants.

On the other hand, even in the minus sulphur plants some of the newly synthesized nitrogenous material might be proteinaceous in nature. The present method did not permit the determination of protein nitrogen, but the data of table III show that most of the organic nitrogenous material of both plus and minus sulphur plants was insoluble in 80 per cent alcohol. No sulphate was added intentionally to the nutrient solution applied to the minus sulphur plants, but there was some present as impurities in the salts used, and some sulphate present in the air might have dissolved in the moist sand. Previous work has shown that the atmosphere of Chicago contains considerable sulphur which is present mainly in the sulphate form (9). So a small part, at least, of the organic forms of soluble nitrogen

which table II shows accumulate in the sulphur deficient plants might represent a step on the way to protein. And of course the newly synthesized protein could be broken down proteolytically as already described.

The preceding considerations indicate that we should not depend entirely on proteolysis to account for the accumulation of the soluble organic nitrogenous fractions in the minus sulphur plants, and may have a bearing in explaining why the carbohydrate situation is different from that usually found in other cases of proteolysis.

CHEMICAL COMPOSITION AND CELL WALL THICKNESS.—The various conditions in plants which may lead to carbohydrate accumulation have already been discussed. It has been noticed repeatedly that accompanying this accumulation the cell walls of the cells in the cortical, phloem, and xylem regions of the stem are thicker than in the case of plants of lower carbohydrate content. NIGHTINGALE (22) found that the minus sulphur tomato plants, which were higher in carbohydrates than the plus sulphur plants, also had thicker cell walls in all parts of the stem. Not much attention was given to the anatomy of the soy bean plants of these experiments, but it was noticed that the stems of the sulphur deficient plants in which starch and hemicellulose accumulated were harder than those of the plus sulphur plants. This was noticed especially in the 1933 plants, and there was greater accumulation of starch and hemicellulose in the plants of this year than in those of 1934 (table IV). Perhaps starch and hemicellulose are more closely related to cell wall thickness than are the sugars, for the sugars were higher in the plus sulphur soy bean plants.

Summary

1. The effects of sulphur deficiency were studied in the soy bean. It took considerable time for symptoms of sulphur deficiency to become apparent, but they were beginning to show in four weeks' time and had become pronounced in six weeks.

2. The main symptoms were the yellow-green color of the leaves, the smaller leaflets, and the thinner stems. These symptoms are similar to those that have been noted for other elements, for example, ni-

4. CROCKER, W., The history of agricultural gypsum. Gypsum Industries Assn. Chicago.

5. ———, The necessity of sulphur carriers in artificial fertilizers. Jour. Amer. Soc. Agron. 15:129–141. 1923.

6. DULEY, F. L., The relation of sulphur to soil productivity. Jour. Amer. Soc. Agron. 8:154-160. 1916.

7. EATON, S. V., Sulphur content of soils and its relation to plant nutrition. BOT. GAZ. 74:32–58. 1922.

8. ———, Effects of variation in day-length and clipping of plants on nodule development and growth of soy bean. BOT. GAZ. 91:113–143. 1931.

9. EATON, S. V., and EATON, J. H., Sulphur in rainwater. Plant Physiol. 1:77–87. 1926.

10. ECKERSON, SOPHIA H., Microchemistry (mimeographed outline of methods).

11. ———, Conditions affecting nitrate reduction in plants. Contrib. Boyce Thompson Inst. 4:119–130. 1932.

12. ———, Influence of phosphorus deficiency on metabolism of the tomato. Contrib. Boyce Thompson Inst. 3:197–217. 1931.

13. HARRISON, C. M., Effect of cutting and fertilizer applications on grass development. Plant Physiol. 6:669-684. 1931.

14. HENRY, W. A., and MORRISON, F. B., Feeds and feeding. Abridged. 5th ed. Henry-Morrison Co., Madison, Wisconsin. 1926.

15. KRAUS, E. J., and KRAYBILL, H. R., Vegetation and reproduction with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149. 1–90. 1918.

16. KRAYBILL, H. R., Plant metabolism studies as an aid in determining fertilizer requirements. Ind. and Eng. Chem. 22:275–276. 1930.

17. MACGILLIVRAY, J. H., Effect of phosphorus on the composition of the tomato plant. Jour. Agr. Res. 34:97–127. 1927.

18. MATTHEWS, A. P., Physiological chemistry, a textbook and manual for students. 5th ed. William Wood & Co., New York. 1930.

19. McHARGUE, J. S., Further evidence that boron is essential for the growth of lettuce. Plant Physiol. 8:305–313. 1933.

20. MILLER, H. G., Further studies on relation of sulphates to plant growth and composition. Jour. Agr. Res. 22:101 114. 1921.

21. NIGHTINGALE, G. T., The chemical composition of plants in relation to photoperiodic changes. Wisconsin Agr. Exp. Res. Bull. 74. 1–68. 1927.

22. ———, Effects of sulphur deficiency on metabolism in tomato. Plant Physiol. 7:565–595. 1932.

23. NIGHTINGALE, G. T., SCHERMERHORN, L. G., and ROBBINS, W. R., The growth status of the tomato as correlated with organic nitrogen and carbohydrates of roots, stems, and leaves. New Jersey Agr. Exp. Sta. Bull. 461. 1–38. 1928.

24. NIGHTINGALE, G. T., SCHERMERHORN, L. G., and ROBBINS, W. R., Some effects of potassium deficiency on the histological structure and nitrogenous and carbohydrate constituents of plants. New Jersey Agr. Exp. Sta. Bull. 499. 1–36. 1930.

25. ———, ADDOMS, R. M., ROBBINS, W. R., and SCHERMERHORN, L. G., Effects of calcium deficiency on nitrate absorption and on metabolism in tomato. Plant Physiol. 6:605–530. 1931.

26. OLSON, G. S., and ST.JOHN, J. L., An investigation of sulphur as a plant food. Washington Agr. Exp. Sta. Bull. 165. 1–69. 1921.

27. PETERSON, W. H., Forms of sulphur in plant materials and their variations with the soil supply. Jour. Amer. Chem. Soc. 36:1290–1300. 1914.

28. PHILLIPS, T. G., et al., The determination of nitrogen in relatively simple compounds. Plant Physiol. 2:205–211. 1927.

29. POPP, H. W., A physiological study of the effect of various ranges of wave length on the growth of plants. Amer. Jour. Bot. 13:706–736. 1926.

30. POWERS, W. L., Studies of sulphur in relation to the soil solution. Univ. California Publ. in Agr. Sci. 5:119–166. 1927.

31. PUCHER, G. W., LEAVENWORTH, C. S., and VICKERY, H. B., Determination of total nitrogen of plant extracts in presence of nitrates. Ind. and Eng. Chem. Analyt. Ed. 2:191–193. 1930.

32. REIMER, F. C., and TARTAR, H. V., Sulphur as a fertilizer of alfalfa in southern Oregon. Oregon Agr. Exp. Sta. Bull. 163. 1–40. 1919.

33. RUSSELL, E. J., Soil conditions and plant growth. 6th ed. Longmans, Green, New York. 1932.

34. STOREY, H. H., and LEACH, R., A sulphur deficiency disease of the tea bush. Ann. Appl. Biol. 20:23–56. 1933.

35. STUART, N., Nitrate and carbohydrate metabolism of young apple trees as affected by excessive applications of sodium nitrate. New Hampshire Tech. Bull. 50. 1932.

36. WEBSTER, J. E., Nitrogen changes in stored alcoholic extracts of plant tissues. Plant Physiol. 8:166–168. 1933.

37. WELTON, F. A., and MORRIS, V. H., Effect of fertility on the carbohydrate-nitrogen relation in the soy bean. Plant Physiol. 5:607–612. 1930.

38. WITHROW, R. B., and RICHMAN, M. W., Artificial radiation as a means of forcing greenhouse crops. Purdue Agr. Exp. Sta. Bull. 380. 1–20. 1933.

MORPHOLOGY AND PHYSIOLOGY OF THE POME LENTICELS OF PYRUS MALUS[1]

HARRY F. CLEMENTS

(WITH NINE FIGURES)

Introduction

The term lenticel, as established by DE CANDOLLE, applies to structures of the stem which typically appear as masses of tissue of the periderm which have enlarged to the point of rupturing the epidermis (3). Its function is regarded as concerned with gas exchange. Were this concept of a lenticel adhered to, the structures on the pome of the apple could scarcely be regarded as such. There are developed no such masses of periderm tissue; in fact it is seldom that periderm activity is associated with these structures. Fruit spots or dots might be more appropriate as descriptive terms were it not that they have been used to denote anything from diseases caused by fungi to insect punctures and even physiological breakdown. Hence, so far as this paper is concerned, the term lenticel will indicate the white, green, or yellowish brown spots common to all apple fruits; for although it does not carry the same connotation with reference to the pome as it does in woody stems, it describes the nature of the pome structures more nearly than do spots, dots, or stomata.

The structure and function of the lenticels of the mature pome have received little attention from investigators. It has been assumed, apparently, that they are similar to the lenticels of woody stems. Because of the possible importance of these structures in gas exchange, and because of their demonstrated importance as infection courts (1) for bluemold and other decay organisms, it was considered worth while to investigate their nature and function.

Historical review

ZSCHOKKE (9, 10) studied the morphology of the apple epidermis including the stomata. He reports that the stomata are completely formed by the time the pome is four weeks old and that at this time they are more or less uniformly distributed over the surface. As the apple increases in size most at the stem end and least at the calyx end, the stomata are distributed by the stretching of the epidermis. In the very young pome from two to ten stomata may be found per square millimeter of surface. ZSCHOKKE (9, 10), BROOKS (2), and KIDD and BEAUMONT (5) were unable to find stomata on the mature fruit and hence concluded that they took no part in gas exchange except in so far as they might act as lenticels. TETLEY (7, 8) gives an excellent account of the development of the stomata and treats of their change to lenticels, suggesting that their formation results from the breaking of stomata caused by the stretching of the epidermis. HAYLETT (4) reports that a lenticel results from a stoma whose substomatal cells become suberized. ZSCHOKKE found that lenticels may result from broken stomata or from scars caused by the falling out of trichomes.

The function of lenticels has generally been assumed to be that of gas exchange. MAGNESS and DIEHL (6) coated apples with paraffin and noted a decrease in the rate of carbon dioxide release and an accumulation of this gas in the intercellular spaces. The opinion that the cuticle is of some consequence in the exchange of gases was expressed by HAYLETT, who further states that the cavity in the apple opening at the calyx end is not an important passageway for gases.

Experimentation

The structure of the lenticel was studied from prepared sections of apple skin cut at right angles to the arc connecting the stem and calyx ends of the apple, as well as sections cut parallel to it. The fruit was taken from local orchards as well as from orchards in the Yakima and Wenatchee valleys, and Prosser, in central Washington.[2] Open and closed lenticels were selected from the apples follow-

[2] The writer is indebted to Dr. KENNETH BAKER who collected the material in the central Washington orchards and who was of constant assistance throughout the work, and to the Department of Plant Pathology for the actual cost of shipment and storage.

ing submergence in a solution of methylene blue, The apples were allowed to remain in the solution for 24 hours, during which time the temperature in the room was allowed to drop some 10° C., causing a mild contraction of the fruit. In this way the dye solution was drawn into the apple slowly and without the possibility of rupturing the lenticels. Lenticels which showed a distinct halo of dye in the parenchymatous tissue below were regarded as open lenticels; those which remained uncolored were regarded as closed; and those which showed a surface adsorption of the dye but without its diffusion into the tissue beneath were at first called partly closed lenticels. These were later found to have the substomatal tissue thoroughly suberized and impermeable to water and dye, and hence were closed lenticels.

The lenticels so selected were fixed in Turtox, imbedded in paraffin, and sectioned. Some of the sections were stained with safranin and gentian violet, although the more satisfactory stain was Sudan III, which stained the cuticle heavily, the suberized tissue lightly, and the unmodified cellulose walls not at all. The sections were mounted in glycerin. Sections were made of lenticels taken from apples of the following horticultural varieties: Jonathan, Winesap, Delicious, Red Delicious, Golden Delicious, McIntosh, Pearmain, Winter Banana, Arkansas Black, Black Stayman, Stayman Winesap, Rome Beauty, Yellow Newtown, Spitzenburger, Grimes Golden, Black Twig, and Starking Delicious.

MORPHOLOGY OF LENTICEL

Pome lenticels are derived from a variety of sources: from stomata which cease functioning as stomata early in the development of the pome; from breaks in the continuity of the epidermis caused by a complete removal of the trichomes characteristically associated with the young apple; and from other epidermal breaks brought about by the inability of the epidermis to keep pace with the expanding inner tissues. These last breaks are usually preceded by a cracking of the cuticle which definitely follows cell boundaries. This is as far as the break goes in many cases and a layer of cutin is formed and no lenticel develops.

Although in most cases the opening into the lenticel is a stoma

(fig. 1), the appearance of the lenticel is the result of change in the subepidermal cells. When the apple is still green and from two to four weeks old, even before the trichomes have dropped, the layers of cells immediately beneath the stomatal aperture become impregnated with a substance that appears to be tannin. This material seems to arise in the cells in which it is found. The cells become so modified that they remain a yellowish brown color while the apple enlarges and develops the red and green colors characteristic of the other hypodermal cells. Thus arises the lenticel or spotty appearance of the apple. That these cells are definitely changed is further indicated by the fact that they do not continue division and growth. In the mature apple, the cells immediately below the stoma appear as though radiating from the substomatal cavity. This evidently is the result of a considerable tension developed by the enlarging fruit; and as these subepidermal cells fail to relieve this tension by the multiplication of cells, they are pulled in such a way as to give the appearance of radiating from the lenticel. That there is much sliding of the subepidermal layers is indicated by the large numbers of intercellular spaces found between these layers in the mature apple (figs. 2, 3). In some instances the pull is sufficiently great to cause a rupture at the point of the stoma (fig. 4). The stoma otherwise remains intact and can be seen with its guard cells in the mature apple, especially if seen in surface view. It is difficult to understand the statements appearing in the literature that the stomata disappear. By far the greater proportion of the lenticels of the apple have uninjured but apparently inactive stomatal apparatus associated with them (fig. 1). In some apples, especially the Grimes Golden, the lenticel is associated with an opening caused by the complete removal of a trichome. This opening is not filled in by the adjacent cells and serves in a way comparable to that of an open stoma. This condition is not common, however. A crack in the epidermis causes the same type of modification of the hypodermal cells as was observed beneath a stoma (fig. 1).

As apples mature the guard cells of the stoma remain permanently open, probably as a result of the stretching which accompanies growth. Thus at first, when the stoma is no longer functional, the developing lenticel is open to the inward as well as to the outward
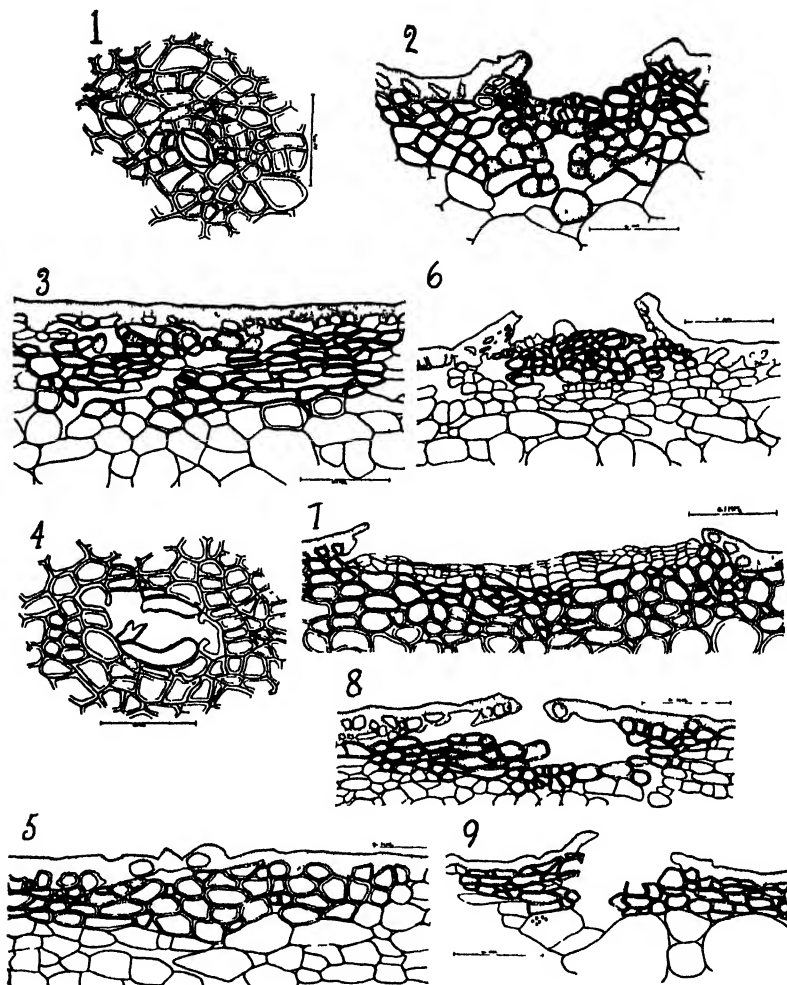
FIGS. 1-9 —Fig. 1, surface view of epidermis over lenticel from Jonathan apple but guard cells are unbroken; a crack in epidermis is also shown (dotted). Fig. 2, section through a closed lenticel from Winesap apple: hypodermal cells exposed are cutinized; cells farther below (dotted) contain tannin. Fig. 3, section through lenticel from Delicious apple: cuticle is uninterrupted across the lenticel. Fig. 4, surface view of epidermis over lenticel from Jonathan apple: guard cells have been broken apart. Fig. 5, section through closed lenticel from Black Twig apple: cuticle has filled the stoma. Fig. 6, section through lenticel from Rome Beauty: suberization of hypodermal cells is taking place but not complete; such a lenticel is still functionally open although it probably would have closed soon. Fig. 7, section through closed lenticel from Rome Beauty apple: phellogen has produced a suberized layer of cells. Fig. 8, section through lenticel from Golden Delicious apple: cutinization has started over exposed hypodermal layer. Fig. 9, section through lenticel from Jonathan apple: a crack has developed which extends from the outside to the large storage cells of parenchyma.

movement of substances. As the apple matures further, more and more of these lenticels become closed functionally although the stomata remain open, until, in such late varieties as the Winesap, nearly all of the lenticels are closed. In fact it is not uncommon to find pomes with only one or two open lenticels.

The closing of lenticels is never associated with mere stomatal closure. The thousand or more lenticels examined never showed the guard cells to be closed; indeed they seemed to be open their maximum amount. Closing of the lenticel is brought about in one of several ways: (1) The cuticle may seal over the stomatal aperture and thus seal the lenticel (figs. 3, 5). (2) The stomatal passage may remain freely open but a cuticle develop over the substomatal layer of cells, thus closing the lenticels to free movements of gases (fig. 2). (3) The stomatal passage may remain freely open and no cuticle develop internally but the substomatal cells become suberized so effectively that even intercellular movement of water or gases is prevented (fig. 6). (4) The stomatal passage may remain freely open, or the epidermal layer with several hypodermal layers may be completely torn away, but the exposed cells develop a phellogen that results in a suberized layer that is quite impermeable both to water and free gas movement; this type is least common (fig. 7).

After the apples were treated in the dye solution, closed lenticels of groups (1) and (2) remained entirely uncolored. Those of groups (3) and (4) showed a surface absorption of the dye with no penetration into the parenchymatous tissue beneath. To test whether or not the lenticels were impermeable to water and gases under pressure, a portion of the skin of the apple (the skin includes the epidermis as well as several layers of hypodermal cells) was removed and placed between two perforated metal discs in such a way that the lenticel was set in the center of the perforation. These discs were then placed in a metal jacket so that air or water pressure could be used. Water was placed over the exposed lenticel and the pressure pump was started. The air was forced against the lenticel with a pressure of 25 pounds. In no case where closed lenticels were used did air bubble through the water even when the pressure was sustained for 30 minutes. Water was then placed inside the container against the lenticel and again the pressure of 25 pounds was developed, but

even after half an hour of such treatment none of the water was forced through. In other words, the closed lenticel appears to be of no significance in the exchange of gas or water. It is probable, of course, that CO_2 can diffuse through the closed lenticel as well as it can through the unbroken cuticle, but this at best is a slow process as will be shown later.

The open lenticels may likewise be of several types: (1) Those in which the stoma remains open with the substomatal tissue incompletely modified or with this tissue completely modified but with large intercellular spaces (fig. 8); this type is found commonly in the very young pomes; (2) Those in which the lenticel had been firmly closed but which have been broken by the tension developed as the apple increased in size. Many times this rupture or tear extends to the very large parenchymatous cells of the storage tissue (fig. 9); (3) Those which are in various stages of repair following such a rupture (fig. 8). It appears that such breaks are repaired rather rapidly in the young apple, but as maturity approaches such rents are less and less easily covered.

NUMBER OF LENTICELS PER APPLE

The number of lenticels found on each apple seems in very broad terms to be a characteristic of the variety, with certain variations caused by the ecological conditions under which the apple is produced. The results of actual counts of the lenticels for the varieties studied are given in table I. Eight to ten apples of each variety were used in these counts.

Table I shows the variations one may expect to find among the varieties. To be sure the variation within a variety is likewise great. The intravarietal variation is proportionally the same in those varieties having a small number of lenticels, such as Winesap, as compared with those having a large number of lenticels, such as Spitzenburger. In Winesap the lenticel counts may average 536, but one apple in ten will show a total count of 450 while another will have as many as 800. Of these, all may be closed or many may be open. These variations are probably explainable on the basis of the ecology of the particular place on a particular tree. As will be seen later, when great care is used in selecting apples from trees, varia-

tion in the number of total lenticels as well as in the distribution of these between open and closed lenticel counts is materially reduced. In such varieties as the Spitzenburger, where the lenticel count is large, the range may be from 1500 to 2500 with correspondingly large variations in the number of closed and open lenticels. Even with this large variation, however, it appears that the lenticel number is something of a varietal characteristic. Inasmuch as the lenticel number seems somewhat related to the size of the apple, one

TABLE I

NUMBER OF LENTICELS PER APPLE

HORTICULTURAL VARIETY	NUMBER OF OPEN LENTICELS (AVERAGE)	NUMBER OF CLOSED LENTICELS (AVERAGE)	TOTAL NUMBER OF LENTICELS (AVERAGE)
Winesap.............	121	414	536
Rome Beauty........	165	388	553
Delicious............	66	737	803
Golden Delicious.....	187	656	843
McIntosh...........	200	651	851
Black Stayman......	46	906	952
Pearmain	121	930	1051
Black Twig.........	185	903	1088
Yellow Newtown.....	78	1106	1184
Starking Delicious. .	92	1221	1313
Red Delicious.......	78	1274	1379
Winter Banana......	170	1909	2079
Spitzenburger........	211	1985	2196

should expect some correlation between it and the variety. In table II are given a few data indicating the relationship between the size of the apple and the number of lenticels within a variety. Thus the smallest five apples average 499 lenticels while the heaviest five average 572 lenticels. Although there seems to be a rather definite relationship, there are exceptions to the generalization. It is probable that this variation is determined by the time the development of the apple has reached its fourth week. Weights of the apple are reported rather than areas, since a close correlation exists between the two and weights are determined with greater accuracy and facility than are areas.

Although there is considerable variation among varieties so far as

the total number of lenticels is concerned, the distribution of lenticels over the surface of the apple is fairly uniform. The varieties studied showed approximately one-third of their lenticels over the stem two-thirds of the apple, with the remaining two-thirds of the lenticels crowded into the calyx one-third of the apple. Generally most of the open lenticels are to be found among the lenticels on the enlarged portion of the mature apple.

To summarize, the morphology of lenticels is much the same in the varieties studied. Actually these structures are not similar to

TABLE II

RELATION OF SIZE OF WINESAP APPLE TO
NUMBER OF LENTICELS

APPLE NO.	WEIGHT (GM.)	NUMBER OF OPEN LENTICELS	NUMBER OF CLOSED LENTICELS	TOTAL NUMBER OF LENTICELS
1	152.5	59	435	494
2	157.2	170	287	457
3	160.5	99	422	523
4	168.5	138	406	544
5	170 0	76	399	475
6	184.5	128	470	598
7	185.7	129	478	607
8	211.5	102	410	512
9	232.9	133	312	545
10	260.0	178	422	600

the lenticels of woody stems. It is only an occasional pome lenticel which shows the development of a distinct phellogen. The large majority of apple lenticels are merely spots underneath a stomatal opening or an opening left by a fallen trichome or break, owing to the modification of the subepidermal cells. These lenticels may be open to mass movements of liquid or gases, or they may be closed in various ways so that neither liquids nor gases can be forced through as such, even under pressure. The number of lenticels per apple varies widely within the variety although within wide limits each variety has its characteristic range. The number of open lenticels per apple ranges from a few in such late varieties as Winesap to several hundred in others.

FACTORS INFLUENCING NUMBER AND TYPE OF LENTICEL

Although the number of lenticels varies within limits character-
istic of the variety, various ecological factors seem to modify these
ranges. It appears, furthermore, that the influences which are
effective must act before the pome has become more than two to
four weeks old. At this time it seems that the stomata as well as
the trichomes are no longer formed, and, as already shown, lenti-
cels are developments of these structures.

Unfortunately apples from variously fertilized plots were not
available, but it was possible to obtain Winesap and Delicious
apples from irrigation plots at Prosser, Washington. One group of

TABLE III

LENTICEL COUNTS OF WINESAP APPLES GROWN UNDER
TWO CONDITIONS OF IRRIGATION

TREATMENT	WEIGHT OF APPLES (GM.)	NUMBER OF OPEN LENTICELS	NUMBER OF CLOSED LENTICELS	TOTAL NUMBER OF LENTICELS
30 inches of irrigation......	188.23	121.2	414.1	535.4
60 inches of irrigation......	202.14	247.4	404.9	652.3

trees was irrigated at the rate of 30 inches of water per season and
another was irrigated at the rate of 60 inches. Lenticel counts were
made on ten apples of the Winesap variety per plot. The results are
recorded in table III. These results indicate that Winesap apples
grown under greater moisture conditions have a greater number of
lenticels than when grown under 30 inches of irrigation. The differ-
ence cannot be accounted for on the basis of area alone. Further,
the difference in the number of lenticels in these two plots is in the
number of open lenticels.

Similar plots of Delicious apples were being conducted under the
two conditions of irrigation. The lenticel counts of these apples are
recorded in table IV.

The data in table IV indicate a response of Delicious apples which
is opposite to that shown by the Winesap apples. Thus even though
the apples receiving more water were larger than the others, the
total number of lenticels on these averaged 69.4 lenticels less than

those on apples grown with 30 inches of water. If the results reported in these tables are significant, then the response of apples with respect to lenticel numbers and irrigation is varietal rather than general.

FACTORS INFLUENCING CLOSING OF LENTICELS

Delicious and Winesap apples were used in this study. Pickings of apples were made at two periods: (1) two weeks before the usual harvest period,—these were called "early picking," and (2) at the usual harvest period,—these were called "prime picking." As soon

TABLE IV

LENTICEL COUNTS OF DELICIOUS APPLES GROWN UNDER
TWO CONDITIONS OF IRRIGATION

TREE	TREATMENT	NO. OF APPLES USED	WEIGHT (GM.)	NO. OF OPEN LENTICELS	NO. OF CLOSED LENTICELS	TOTAL NO. OF LENTICELS
B-8.........	30 inches	15	255.4	141.2	1343.2	1484.4
C-8.........	30 inches	15	235.5	169.3	1351.3	1520.6
D-8.........	30 inches	18	236.2	110.2	1192.4	1302.6
Average of 48 apples		....	242.4	142.2	1295.6	1435.8
B-12.........	60 inches	15	282.9	132.0	1244.1	1376.1
C-12.........	60 inches	15	283.2	111.8	1237.8	1349.6
D-12.........	60 inches	15	279 9	116.6	1256.9	1373.5
Average of 45 apples		....	282.0	120.1	1264.3	1366.4

as the apples were brought into the laboratory, some were subjected to the dye treatment and lenticel counts were made. Some were placed in ventilated containers at a temperature of 30° C. and relative humidity of 5–8 per cent. These apples were kept in this container for a week. Other lots were placed in other containers the temperature of which fluctuated between 10° and 15° C. and the humidity between 60 and 70 per cent. Still other lots were placed in desiccators over $CaCl_2$ at a temperature of 25° C. After the experimental period, the apples were subjected to the dye treatment and lenticel counts were made. The results are given in table V.

Winesap apples of both early and prime pickings were studied in a manner similar to the preceding series. The results are recorded in table VI.

It is apparent from these two tables that lenticels can be closed by any treatment which favors dehydration. Thus when apples are placed in a desiccator over CaCl, and at moderate temperature, nearly all of the lenticels are closed. Prime picked apples are less responsive than those picked earlier. It is to be noted that this closing effect obtains while the apples are still on the tree between the time of the early and prime pickings. The apples of the prime picking have about one-fourth the open lenticels of the previous picking.

TABLE V

EFFECT OF TEMPERATURE AND HUMIDITY ON LENTICEL
OF DELICIOUS APPLES

TREATMENT	NUMBER OF APPLES	NUMBER OF OPEN LENTICELS PER APPLE
EARLY PICKING		
Untreated apples....	28	84 4
Apples kept at 30° C. and 8–10% humidity for 1 week	47	4 9
Apples kept in desiccator at 25° C. for 1 week............... ...	12	1 8
Apples kept at 10°–15° C. and 60–70% humidity for 1 week......	65	52 9
PRIME PICKING		
Untreated apples..	31	49 3
Apples kept at 30° C. and 8–10% humidity for 1 week..........	24	23 04

This effect does not continue after the prime picking date, for if the apples are left on the tree they develop a large number of cracked lenticels or parts of the epidermis. This may be a response to water relations, for after some of the fruit is picked or has fallen, those apples remaining may absorb quantities of water sufficient to cause enlargement of the fruit, resulting in the cracking of the epidermis.

Another series was studied to determine whether apples which had been in storage until December 15 were still capable of closing their lenticels. Delicious apples were used. One lot was treated with dye solution as soon as it was taken from storage, and 51 apples of this lot averaged 48.04 open lenticels. A second lot was placed in a container kept at 30° C. and 8–10 per cent humidity, and 25 apples in this lot averaged 51.4 open lenticels. A third lot was treated as

lot 2 for one week and then was transferred to another container with a temperature of 15° C. and 60–70 per cent humidity for six weeks and 43 apples so treated averaged 49.9 open lenticels. A fourth lot was taken from storage and kept at 15° C. and 60–70 per cent humidity for six weeks, and 48 apples so treated averaged 33.7 open lenticels.

Thus it appears that, in storage, the senescence of apples increases to the point where a marked decrease in the reactivity of lenticels is

TABLE VI

EFFECT OF TEMPERATURE AND HUMIDITY ON LENTICELS
OF WINESAP APPLES

TREATMENT	NUMBER OF APPLES	NUMBER OF OPEN LENTICELS PER APPLE
EARLY PICKING		
No treatment...	62	49 5
Apples held 2 weeks at 30° C and 5–8% humidity	65	5 4
Apples held 2 weeks at 25° C. in desiccator over CaCl₂	12	o 66
Apples held 2 weeks at 15° C. and 60–70% humidity	69	33 72
PRIME PICKING		
No treatment...	47	13 6
Apples held 2 weeks at 30° C. and 8–10% humidity	95	3·5
Apples held 2 weeks at 15° C. and 60–70% humidity	73	10.1

to be noted. It is only in lot 4 that any significant change has taken place. Further, no change takes place during the storage period of these apples. The apples placed in storage were of the same lot as those reported in table V of the prime picking series. When these apples went into storage they averaged 49.3 open lenticels and when they came out they averaged 48.04.

To summarize, it appears that the less mature apples respond more rapidly to treatments which induce lenticel closure than do mature apples. After a period in storage, Delicious apples fail to respond to treatment except after prolonged periods. The same type of closing takes place on the tree up to maturity, after which it appears that the lenticels are broken open by the enlarging fruit.

RATE AND COURSE OF GASEOUS DIFFUSION FROM APPLES

Delicious apples taken from storage were used in these studies. They were taken from $1°$ C. and transferred as rapidly as possible to a chamber in a gas chain held at $25°$ C. Carbon dioxide-free air was drawn through the chamber and the carbon dioxide released by the apple was collected in absorption bulbs charged with KOH solution after the gas was dried by sulphuric acid. The air was moved through rapidly enough to prevent a "piling up" of the gas in the chamber. Weighings were made once each hour. This was continued until the hourly losses showed no appreciable variations.

It was necessary to determine the time required for the temperature of the apple to reach equilibrium with the warm outside temperature. This figure was obtained by thrusting a thermometer bulb into the flesh of the apple and making readings until the temperature of the apple was within half a degree of the air temperature. A typical series follows: at 4:00 P.M. the temperature of the apple was $1.0°$ C.; at 4:20, $3.0°$ C.; at 4:31, $4.0°$ C.; at 4:37, $5.0°$ C.; at 4:57, $9.0°$ C.; at 5:19, $12.5°$ C.; at 6:30, $20.0°$ C.; at 6:53, $22.5°$ C.; and at 7:50, $24.0°$ C. The air temperature was $24.5°$ C. Thus in 3 hours and 30 minutes the internal temperature of the apple was within a half degree of the temperature of the room.

Five Delicious apples were used in this test. It was hoped that after the gas determinations were complete and the number of open lenticels determined that sufficient variation in the number of open lenticels would exist to throw some light on the importance of these structures in gaseous diffusion. Fortunately one apple had only two open lenticels and another had 87. The first apple weighed 319 gm., the second 333 gm. CO_2 loss from these apples was practically identical. The amount of the gas lost during the test was slightly more than was calculated to give a saturated solution of it in the apple at the beginning of the experiment. The time necessary for the gas to come to equilibrium with the new temperature was 52 hours in the case of the apple with two open lenticels, and 49 hours in the case of the apple with 87 open lenticels. The CO_2 release in all the apples studied began very slowly (0.0008 gm. per hour) and rose more or less uniformly until a maximum of 0.0221 gm. per hour was reached at the end of 24 hours. The drop to equilibrium,

in about 24 hours more, was nearly a mirror image of the rise. It is obvious from these results that open lenticels play a very small part in the exchange of gases. It appears likely that the gas moves through the cuticle directly. If this is true, one might expect a slow movement through the closed lenticels as well, but surely these structures cannot be regarded as special organs for this function.

The importance of the cavity leading from the stem tip to the calyx end of the apple in gaseous exchange has been variously regarded. HAYLETT reports that it is insignificant in this regard. However, apples were studied by taking them from storage and plunging them into water heated to 60° C. Bubbles of the gas formed over the surface of the apple with no preference shown to lenticels, unless these were obviously open. There was vigorous bubbling from the calyx end of the apple. Whether this is a fair test or not is a matter for conjecture, but it is probable that when the gas pressure within the apple is large (as it would be if the apple is changed from 1° to 60° C.), the course would be as just noted. Further work will be done to determine the course during normal pressures.

When Winesap apples which averaged only 96 gm. per apple were used instead of Delicious, equilibrium time averaged 51 hours. Thus although these apples were approximately one-third the size of the Delicious, the time for equalization of gas pressures was approximately the same. It must be understood, however, that resistance to the escape of gases offered by the two varieties is probably quite different.

It is to be noted, finally, that moisture is lost from open lenticels from their immediate environs. When apples are allowed to stand in dry air for a few days, some lenticels are to be found in small pitlike depressions. These lenticels are open and the pit is formed as a result of water loss from the parenchymatous cells beneath. It is also probable that carbon dioxide produced in the vicinity of an open lenticel is lost through it.

Summary

1. The development of the lenticels of 18 horticultural varieties of apples is traced. Lenticels may be open or closed depending on the character of the hypodermal cells. These may be cutinized or

suberized and thus rendered closed to the free movement of gases or liquids. Lenticels may also be closed when the stoma associated with the lenticel is closed over by means of the epidermal cuticle. Lenticels may be open when the hypodermal cells are unmodified or when the modified cells have been torn apart.

2. The total number of lenticels per apple is within certain wide ranges characteristic of the variety. The number may range from 450 to 800 in the case of Winesap apples and from 1500 to 2500 in the case of Spitzenburger apples.

3. The number of lenticels per apple may be varied by varying the amount of water available to the plant during the early development of the apples. This reaction is varietal rather than general. The Winesap apple when given more water produced more lenticels per apple than when grown with less water. The Delicious apples when given more water actually produced fewer lenticels even though the apples were larger.

4. Lenticels may be closed by processes which favor dehydration of the outer tissues of the apple. While the apples are still immature, they respond more completely to such treatment than do mature apples. After apples have been in storage for 6–8 weeks, they respond only after prolonged treatments.

5. Carbon dioxide gas within the apple escapes with equal speed whether the apple has many or few open lenticels. The period necessary for the gas to come to equilibrium with new temperatures is 49 to 52 hours.

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LITERATURE CITED

1. BAKER, K. F., and HEALD, F. D., The importance of lenticel infection of apples by *Penicillium expansum*. State Coll. Washington Agr. Exp. Sta. Bull. 264. 1932.
2. BROOKS, CHARLES, The fruit spot of apples. Bull. Torr. Bot. Club 35:423–456. 1908.
3. HABERLANDT, G., Physiological plant anatomy. MacMillan and Co., New York.
4. HAYLETT, D. F., Studies on the effect of humidity on the respiration of plant parts. Cornell University Thesis. 1925.

5. KIDD, M. N., and BEAUMONT, A., An experimental study of the fungal invasion of apples in storage with particular reference to invasion through lenticels. Ann. Appl. Biol. 12:14–33. 1925.

6. MAGNESS, J. R., and DIEHL, H. C., Physiological studies on apples in storage. Jour. Agr. Res. 27:1–38. 1924.

7. TETLEY, URSULA, A study of anatomical development of the apple and some observations on the pectic constituents of the cell wall. Jour. Pomology and Hort. Sci. 8:153–172. 1930.

8. ———, The morphology and cytology of the apple fruit, with special reference to Bramley's seedling variety. Jour. Pomology and Hort. Sci. 9:278–297. 1931.

9. ZSCHOKKE, A., Ueber den Bau der Haut und die Ursachen der verschiedenen Haltbarkeit unserer Kernobstfrüchte. Landw. Jarhesb. Schweiz. 11:153–196. 1897.

10. ———, Die Ursachen der verschiedenen Haltbarkeit unserer Kernobstfrüchte. Jahresb. Deutsch-schweizerischen Versuchstation and Schule f. Obst.-Wein- und Gartenbau in Wadenweil. 1894–1895. 5:56–57. 1896.

MORPHOGENETIC STUDIES ON THE INFLORESCENCE OF COTTON

ULYS ROY GORE

(WITH FIFTY-THREE FIGURES)

Introduction

A number of papers on the fruiting behavior of cotton have appeared (3, 1, 17). Work has been done also on the influence of ecological factors on fruiting behavior (13), the arrangement of parts in the cotton plant (4), regularity of blooming (16), lint development (7), microsporogenesis and chromosome numbers (2), megasporogenesis (9), and root stem transition (21). Many genetic studies dealing with inheritance of floral characters, fruiting behavior, and vegetative characters have been summarized by KEARNEY (12). The purpose of the present paper is to describe the ontogeny and development of the primary axis (main stem) and of the floral axis (fruiting branch), together with the developmental morphology of the flower and its vascular anatomy. An attempt is made also to interpret the androecial column on the basis of ontogenetic studies.

Materials and methods

Material from cotton plants growing near Fayetteville, Arkansas, of the Pima, Sea Island, and Mebane varieties, was collected at various intervals throughout the summer of 1933. This was fixed and preserved in a modification of Navashin's solution consisting of equal parts of A (7 cc. glacial acetic acid, 1 gm. chromic acid crystals, 92 cc. distilled water) and B (30 cc. formalin and 70 cc. distilled water), mixed at the time of using and left in this solution until needed for study. For the anatomical details the material was imbedded following the n-butyl alcohol technique, sectioned at 15 μ, and stained in safranin-gentian violet-orange combination. Both longisections and transections were made of the different bud clusters, but transections proved to be more valuable for interpreting the

method of development of the axes as well as for the vascular details of the flower.

It was found most convenient for a study of developing axes and floral ontogeny to make observations under a wide-field binocular microscope. For this study the subtending leaves with stipules were dissected away from the stem tip, exposing the young primordia; then, following the method used by LUYTEN (15), the primordia were stained with strong IKI, which facilitated the observations. Plants grown in the greenhouse in the spring of 1933 failed to produce more than a single flower to a fruiting branch and hence were not used in these studies.

Investigation

PRIMARY AXIS

DESCRIPTION.—The main stem consists characteristically of a single indeterminate primary axis from which the branches arise. With the exception of the cotyledons, the leaves occur on the main stem in a regular 3/8 spiral arrangement. Examples of variation from this arrangement are found in hybrid stocks, which may have a 1/3, 2/5, or 5/13 spiral arrangement. The Old World species have a 1/3 spiral leaf arrangement (4).

Two clearly distinct types of branches, differing in form and function, arise in the axil of the leaves on the main stem (3, 4). These are (1) vegetative branches similar in all respects, morphologically and functionally, to the primary axis; (2) fruiting branches which are different in form, function, and method of development.

At a node on the main axis in Mebane, the following structures are generally found: a leaf with stipules, an axillary branch either fruiting or vegetative, and an accessory dormant bud. In Pima and Sea Island this accessory bud usually develops, but is soon abscissed. This accessory bud, when located at the base of a vegetative branch, is termed "extra-axillary" by COOK (3) and KING (13); and when located at the base of a fruiting branch, "axillary." According to GRAY (10) and GOEBEL (8), buds are normally either terminal or axillary, are usually single, and appear in the axil of the leaf. When more than one bud appears in the leaf axil (10) it is due to the fact

that the true axillary bud or primordium has by branching given rise
to one or more extra buds.

ONTOGENETIC STUDIES.—The main stem and the vegetative
branches are developed by continued activity of the terminal bud of
each respective axis. Leaves with stipules and axillary bud primor-
dia are developed in acropetal succession at each node from a ter-
minal meristem. This method of growth is monopodial in character
as contrasted with sympodial growth. The terminal growing point
is surrounded and protected by the primordia of several leaves and
stipules, these structures being covered with hairs which afford com-
plete protection to the developing primordia.

The early development of the leaf and stipules proceeds as follows:
the leaf primordium and stipule primordia arise practically simul-
taneously from the apical meristem of the primary axis (figs. 1, 2).
The leaf primordium is rounded and collar-like at first, later tapering
somewhat up to the rounded tip. As the embryonic leaf grows up-
ward it becomes bluntly pointed and assumes the shape shown in
figure 2. There is little evidence of differentiation into petiole and
blade at this stage, except the slight beginning of the plicate folding
characteristic of the lamina of a young leaf. No epidermal hairs are
present at this stage, although they begin their development early.
The third young leaf from the tip is completely covered with them.

The tip of this first protuberance becomes the median lobe of the
palmate leaf; its base develops into the petiolar region. There soon
develops on the slightly incurved margins of this primordium two
pointed protuberances located about halfway between the tip and
its base (fig. 1). These are the primordia of the two lateral lobes of
the leaf. The young leaf, consisting of three lobes, now proceeds to
develop in length; continued inward plicate folding, as the lobes in-
crease laterally in size, forms a cuplike portion (figs. 2, 3). If addi-
tional lobes occur they arise in the same way and are similar in all
respects (figs. 4, 5). Seven lobes is the usual number for leaves on the
main axis, while on the fruiting branches of Pima and Sea Island
varieties the leaves usually have three. In Mebane five-lobed leaves
are commonly found on the fruiting branches. Soon the tips of the
individual lobes meet and the lamina develops rapidly. The main
veins appear as definite ridges on the abaxial surface of the leaf and
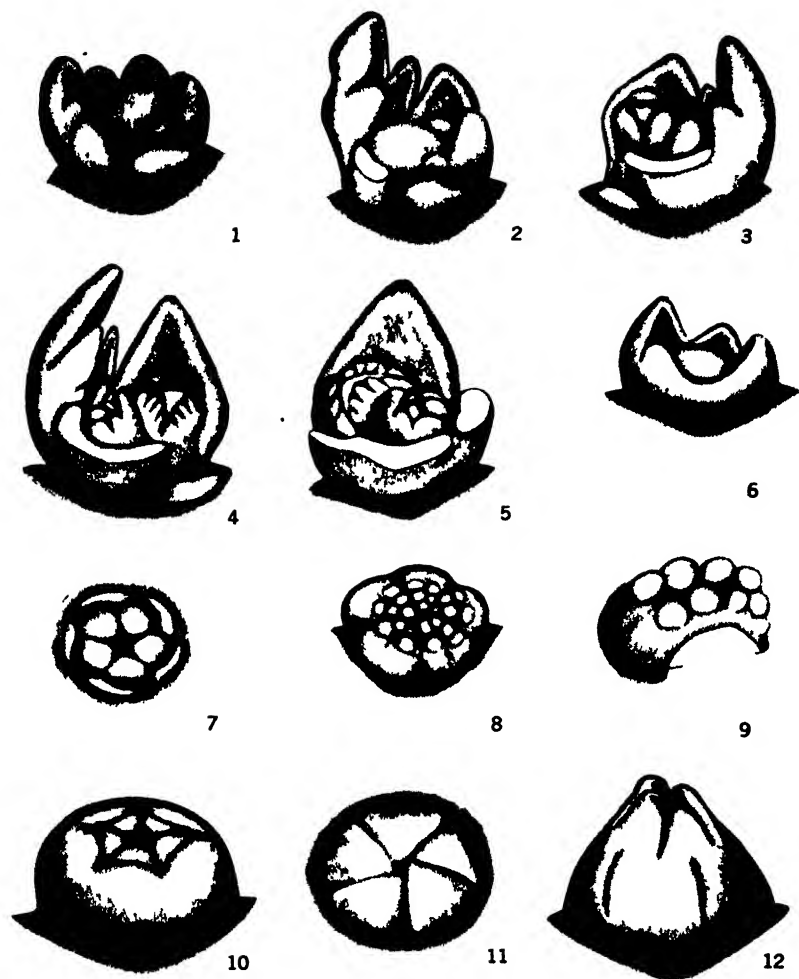
FIGS 1-12 — Fig 1, growing point of cotton showing early stages in ontogeny of leaf and stipules, axillary bud, lobes of young leaf, and general topography of tip of main axis, drawn from above and slightly to one side, Pima Fig 2, later stage in leaf ontogeny, one stipule cut away, Mebane Fig 3, general topography of fruiting branch terminal, origin of bracts on flower primordium and new axillary primordium, Sea Island Fig 4, later stage in ontogeny of sympodial axis showing slightly older floral bracts; Pima Fig 5, later stage than fig 4 showing origin of new fruiting branch terminal between leaf and flower primordium, Mebane Fig 6, axillary bud of fruiting branch showing bractlike structure, growing point, leaf and stipule primordia of second node; Mebane Fig 7, early ontogenetic stage of staminal column, petal primordia well differentiated and showing alternate staminal column lobes; Sea Island Fig. 8, later stage in ontogeny of staminal column showing stamen primordia in two rows on each ridge; Pima. Fig 9, one of the five lobes of developing staminal column with stamen primordia, under greater magnification than fig 8; Pima. Fig. 10, young calyx with bracts removed; Mebane Fig 11, developing petals, Pima. Fig. 12, young pistil; Mebane.

are very thick in proportion to the rest of the leaf. The venation is palmate.

Simultaneously with or shortly after the initial leaf primordium becomes distinct, two stipular flanking protuberances arise (fig. 2). These are pointed and concave at first, but as they grow they widen out from the base somewhat, later become toothed, fold inward very slightly, and keep pace with the growth of the leaf. They are not folded or plicate as is the young leaf but remain more or less flattened, their interlocking hairs holding them tightly appressed to the leaves.

FRUITING AXIS OR SYMPODIUM

DESCRIPTION.—Casual examination of a fruiting branch shows that the flowers are not in the axil of a leaf (fig. 13), but appear to stand opposite the adjacent leaf. The internodes of this branch are slightly zigzag and the leaves appear as if alternately arranged. Detailed examination shows that this arrangement is the result of sympodial development. A sympodium, according to GRAY (10) and GOEBEL (8), consists of a series of seemingly superposed internodes. Actually each internode (or several internodes) belongs to succeeding generations of axes. Such a sympodium is developed more or less as follows: From the axil of a leaf on a given axis there arises a new axis which by its growth pushes the terminal portion of the axis from which it arose to one side so that this once terminal portion may come to appear as if lateral in origin. Many examples of sympodial types of development are to be found (8, 10) in the Caryophyllaceae, Malvaceae, Solanaceae, Linaceae, etc.

The fruiting branches of all known cottons are now recognized as sympodial (22). This type of inflorescence was earlier termed a false raceme by GOEBEL (8), and was so applied to cotton by BALLS (1). LEAKE (14) was among the first to group the Indian cottons on the basis of their secondary branches into monopodial and sympodial forms. He also observed that monopodial branches are usually ascending while sympodial branches are spreading. COOK (3) opposed the sympodial interpretation and termed the cotton inflorescence as either an "extra-axillary" branch or simply a fruiting branch. COOK and MEADE (4) stated that the behavior of cluster cottons may indicate that the fruiting branches have a sympodial method of growth.

They speak also of the abortion of the terminal bud of the fruiting branch.

A single segment of a fruiting branch consists of an internode, a
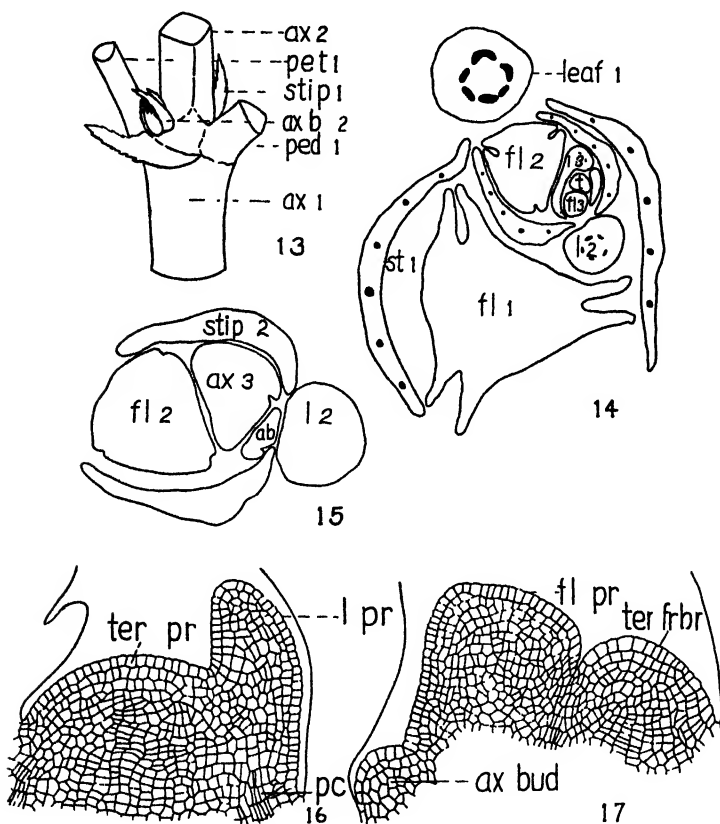
FIGS. 13 -17.—Fig. 13, sketch of portion of fruiting branch of Mebane showing parts of two sympodia; termination of axis 1 is the flower whose pedicel is shown, together with leaf and stipules: *ax 1, 2*, axes 1 and 2; *pet 1*, petiole of axis 1; *ped 1*, pedicel of flower; *ax b 2*, axillary bud developed from axis 2. Fig. 14, outline of transection of terminal bud of fruiting branch showing three sympodia in cross section and terminal for fourth axis; axillary fruiting branch buds not shown: *fl, 1, 2, 3, L 1, 2, 3, st 1* and *t*, flower, leaf, stipule, and terminal for respective sympodia. Fig. 15, lower level on sympodia 1 and 2, also greater magnification; axillary bud of axis 3 is shown: *ax 3*, axis 3; *ab 3*, axillary buds axis 3. Fig. 16, longisection cell drawing of terminal of main axis; Mebane: *ter pr*, terminal primordium of axis; *l pr*, leaf primordium; *pc*, procambium. Fig. 17, longisection cell drawing of terminal fruiting axis: *fl pr*, flower primordium; *ter fr br*, terminal fruiting branch; *ax bud*, axillary fruiting branch bud.

leaf with stipules, the flower bud, and two axillary buds one of which remains dormant and becomes the axillary bud of the fruiting branch while the other continues the secondary axis. The fruiting branch of cotton is made up of a series of these segments which gives to the entire structure a jointed appearance.

The importance of sympodial development from the economic point of view is related to two well known facts. First, flowering and fruiting are wholly dependent upon the production of sympodia. Certainly the number of potential flower buds produced, among other factors, influences the yield of cotton. Secondly, the time at which production of sympodia begins, whether early or late, determines earliness or lateness of fruiting. The early fruiting and maturing varieties begin the production of sympodia early, some even at the first or second node, and no vegetative branches are produced in some varieties. Pima produces a number of monopodial divergences before any sympodial divergences appear.

ONTOGENETIC STUDIES.—The first evidence of the development of a fruiting branch is a primordium which occurs in the axil of a leaf on the main stem at the second or third node back from the apical meristem (fig. 2). This axial primordium is early differentiated into two separate growing points: one grows faster and becomes the fruiting branch primordium; the other grows slowly and develops the axillary bud on the main axis, which is homologous with the axillary bud of the fruiting branch. The fruiting branch primordium becomes raised at three points where the first leaf and stipules are to arise. At this stage a single primordium exists which resembles the apical primordium of the main stem. After the leaf and stipule primordia are definitely differentiated it begins to grow away from the subtending leaf and is directed away at an increasing angle. It becomes bluntly conical in shape and projects beyond the subtending stipules (fig. 4), continuing its development into a flower bud. At this same time a zone of tissue becomes active between the leaf and the primordium of a flower (fig. 5). It soon develops two unequal-sized growing points, the smaller of which gives rise to the axillary bud; the larger one continues the fruiting branch by developing a leaf and stipules for the next axis. By a repetition of this process the fruiting branch comes to consist of a series of axes, each axis made up of a single in-

ternode and each terminated by a flower. Cottons in which a sympodium consists of more than one internode are exceptional. All cultivated American species have a single internode to each sympodium or axis (**22**). Early in the development of the fruiting branch, when the first fruiting axis is being formed, it is pushed to one side so that the developing structures are projected to one side of the leaf on the main stem. Thus a fruiting branch is more nearly horizontal with the primary axis and grows out either to the right or left of the subtending leaf on the main stem.

The arrangement of the succeeding axes is shown in figures 14 and 15, which are from transections of the tips of young fruiting branches of Mebane. Here are shown the parts of three successive axes. The older proximal axes project beyond the younger distal ones so that the petioles of the leaves on axes 2 and 3 are shown in transection. In a longisection of a tip of a fruiting branch (fig. 17) may be seen the flattened floral primordium, the axillary bud primordium, and the new terminal growing point located between the leaf and flower primordium.

AXILLARY BUD OF FRUITING BRANCH

The behavior of the bud occurring in the axil of a leaf on a fruiting sympodium has been investigated by KING (**13**) for Pima and Upland cottons. No detailed study of the ontogeny or the morphology of these structures is available, but they have been described as developing short vegetative branches by COOK (**3**) and as single floral buds without any subtending leaves or stipules by KING (**13**).

By dissecting out the successive axillary buds on a fruiting branch it is possible to get all stages in their ontogeny and development, from the earliest primordium to the flower bud, leaf, and stipules. Axillary buds from fruiting branches of Pima, Sea Island, and Mebane were studied. Since Mebane has the greatest variation in structure it will be described first. It has its origin from the axillary primordium of the fruiting sympodium as already described. The first divergence from the axillary bud primordium is a bractlike structure, unattended by stipules, possibly representing a reduced leaf and stipules. There next arise a leaf and stipules for the second node, followed by the turning aside of the growing point and the develop-

ment of a sympodium. The new terminal primordium develops a
monopodial axis at the next node, however, and at a succeeding node
another sympodium may be developed. This alternate production of
a sympodial axis and a monopodial axis may continue until three or
four flower primordia are produced. As pointed out by KING (13),
these axillary bud structures on Upland varieties usually remain dor-
mant, until late in the season when proper conditions arise they de-
velop branches; but only one of the flower buds functions. Figures
18–24 show consecutive cross sections of this bud in Mebane.

In Pima and Sea Island these axillary fruiting branch buds arise
exactly as in Mebane. Their further development, however, is dif-
ferent. They are with respect to their mode of development similar
to sympodia of the fruiting branches of these respective varieties.
Each axis has its complement of leaf, stipules, flower bud, axillary
bud, and terminal. The vascular structure of this axillary fruiting
branch bud is like that of the fruiting axis and these studies show
that in Pima and Sea Island most of these branches consist entirely
of sympodia (figs. 25–33).

In Pima and Sea Island these axillary buds do not usually remain
dormant, but develop shortly after they are formed, lengthening the
first internode to 0.5 inch or longer. They usually do not grow long
before they shrivel up and are abscissed. This confirms KING's work
on Pima. In Mebane these buds may develop a flower when the
extra-axillary flower bud is shed, or late in the season they may pro-
duce short axillary branches. The causes for differences in behavior
of these axillary fruiting branch buds of the different varieties are
not known and need further investigation.

COTTON FLOWER

DESCRIPTION.—The general features of the cotton flower and the
arrangement of its various organs are described by BALLS (1), COOK
and MEADE (4), and ROBBINS (18), so that only a brief summary is
necessary here before taking up the ontogeny of the individual floral
organs.

The flowers in the species studied are extra-axillary, terminal, and
solitary, although they may appear as if arranged on alternating
sides of the fruiting branch (fig. 13). There are five whorls or cycles

of organs: the first whorl consists of an epicalyx of three relatively large leaflike bracts; the next whorl or true calyx consists of five unequally lobed, mostly undiverged sepals; the third whorl, the corolla,
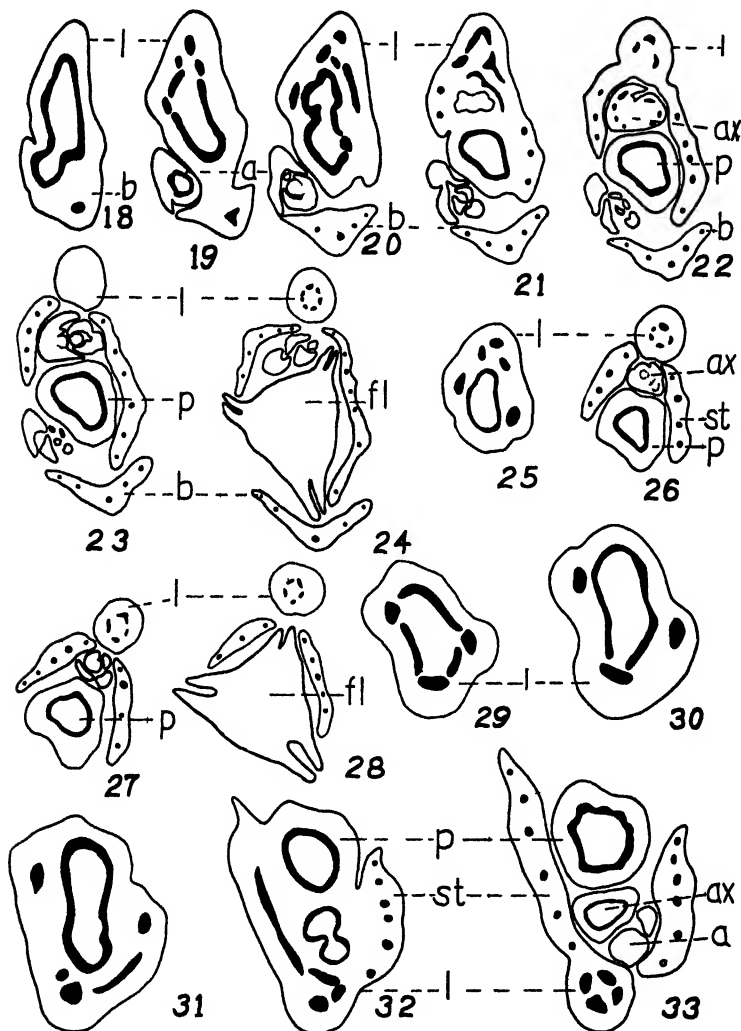
FIGS. 18-33.—Figs. 18-24, successive transections of axillary fruiting branch bud (Mebane) showing two types of axes; vascular details shown in black. Figs. 25-28, successive sections of same for Sea Island. Figs. 29-33, successive sections of same for Pima; note sympodial nature of bud.

consists of five obcordate petals, white in Mebane and yellow in Pima and Sea Island, with a purple spot on the claw of the petal in the latter variety. The petals are convolute in the bud and are held tightly folded together by epidermal hairs. The fourth whorl or androecium consists of a staminal column bearing ten more or less double rows of stamens with reniform two-loculed anthers. The last whorl or the pistil is made up of from three to five undiverged carpels. The fruit is a three to five loculed capsule or boll, dehiscent by splitting along the dorsal sutures of the carpels. The seeds are covered with lint hairs which are elongated epidermal cells of the outer integument (7).

ONTOGENETIC STUDIES.—The young flower becomes evident with the appearance of a bract primordium on the floral meristem. This first primordium is smooth margined, and appears opposite the leaf (fig. 3). The next two bracts follow in succession and are similar in shape to the first. The three bracts grow rapidly in size, and lobes, which become the teeth of the mature bracts, are evident early. The floral bud is now triangular in shape.

When the bracts reach the stage shown in figure 5, the primordium of the calyx arises. This primordium consists at first of an undulating collar or meristematic zone which, as it grows upward, grows more rapidly at five definite points. These points become the tips of the unequally five lobed gamosepalous calyx. The rounded lobes of the calyx become folded over and inward (fig. 10), and for a time cover almost completely the remaining developing floral organs. Both the bracts and calyx are persistent, remaining green until the boll ripens.

After the sepal primordia arise there remains a rounded mass of meristematic tissue at the center. With subsequent growth of this · mass its outer margin becomes raised, leaving a depression. This now collar-like ring is the beginning of the common stamen-petal zone or primordium (fig. 10). The ring soon becomes noticeably elevated at five points on its rim, at an earlier stage than that shown in figure 10, and five lobes arise simultaneously on its lateral face. These thickened regions alternate with the sepal lobes and are the petal primordia.

The five petal primordia become flattened as they grow out from

the zonal meristem, increasing in size both laterally and vertically. An apical notch occurs when the petal is very young. The petals grow more at the top than at the base, as seen in figure 11, and so become convolute in the bud. Continuing their growth in height they soon push up and overtop the young staminal column. They
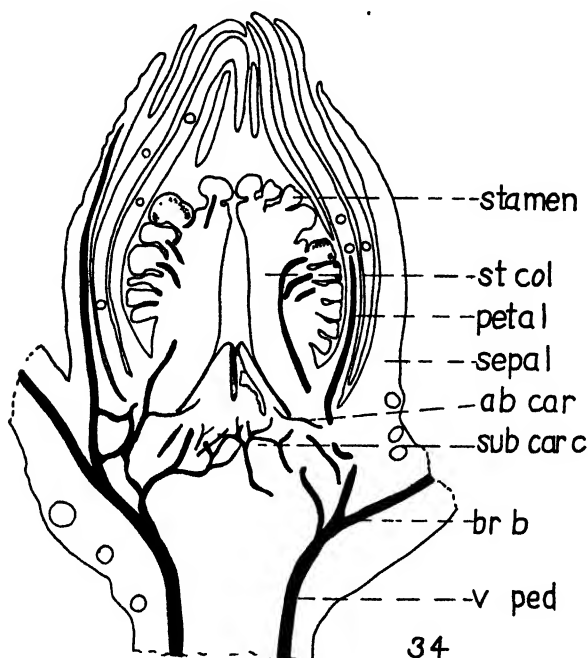
FIG. 34.—Nearly median longisection flower bud of Mebane showing vascular supply to various organs at this stage of development: *v ped*, vascular supply to pedicel; *br b*, bract bundle; *sub car c*, subcarpellary complex; *ab car*, abaxial carpellary bundle; *st col*, staminal column.

overlap, either to the right or left, and are held together in the bud by interlocking hairs. After becoming folded over one another, a further upward growth brings their tips out beyond the sepals as a conical-shaped structure, with the sepal tips of the calyx cup tightly appressed around the corolla (fig. 34).

It was just stated that the petals and stamens develop from a common zone of embryonic tissue. The first evidence of divergence of the staminal column occurs before or about the time petal primordia

are diverged, when five distinctly higher points appear on the upper and inner margin of this ring, and occupy most of the rim, with slight depressions between each of them (fig. 7). The inner edges now appear most active and grow inward for a time. On each of these five primordial lobes there begin to appear rounded primordia of stamens directed toward the center on the inner face of each lobe. Figure 8 is of a developing staminal column of a slightly later stage, for here ten rows or ridges of stamen primordia can be seen, with deeper furrows between pairs of them. The original five lobes shown in figure 7 can still be recognized, however. It is significant that the staminal column lobes alternate with the petals at this stage, but with later growth and twisting of the petals the staminal column lobes appear opposite the petals. Soon after the first spherical stamen primordia arise, other primordia appear on top and down the outside face of each lobe of the staminal column (fig. 9), until ten definite rows are formed. Each of these ten rows of stamen primordia, except the bottom members of each row, divides one or more times. Multiplication of stamens by division of existing primordia produces a large number of stamens, from 50 to 125 or more in the varieties studied. Branching of stamen primordia occurs at the tip of the staminal column as well as along the sides. This multiplication of the members of a whorl of floral organs is termed "deduplication" or "chorisis" (8, 10), and occurs in the Tiliaceae and Hypericaceae (5) as well as in the Malvaceae. The young anthers are at first spherical, but later become two lobed and reniform in shape. The short filaments bearing the capitate anthers grow little more until a few days before flowering, when they increase greatly in length. The fully formed stamens in a flower bud have the appearance of a rounded compact mass of anthers. The top of the staminal tube remains definitely five lobed at maturity.

In the cavity in the center of the axis which has been overtopped by the staminal column, the carpel primordia arise. They are first evident as a zonal meristem slightly raised at three to five definite points. These points appear very early and become the stylar projections. Their further growth is extremely slow in the early stages as compared with the growth of the rest of the young pistil (fig. 12). The upward growth of the rim of the ovary brings the styles to project into the staminal tube at an early age.
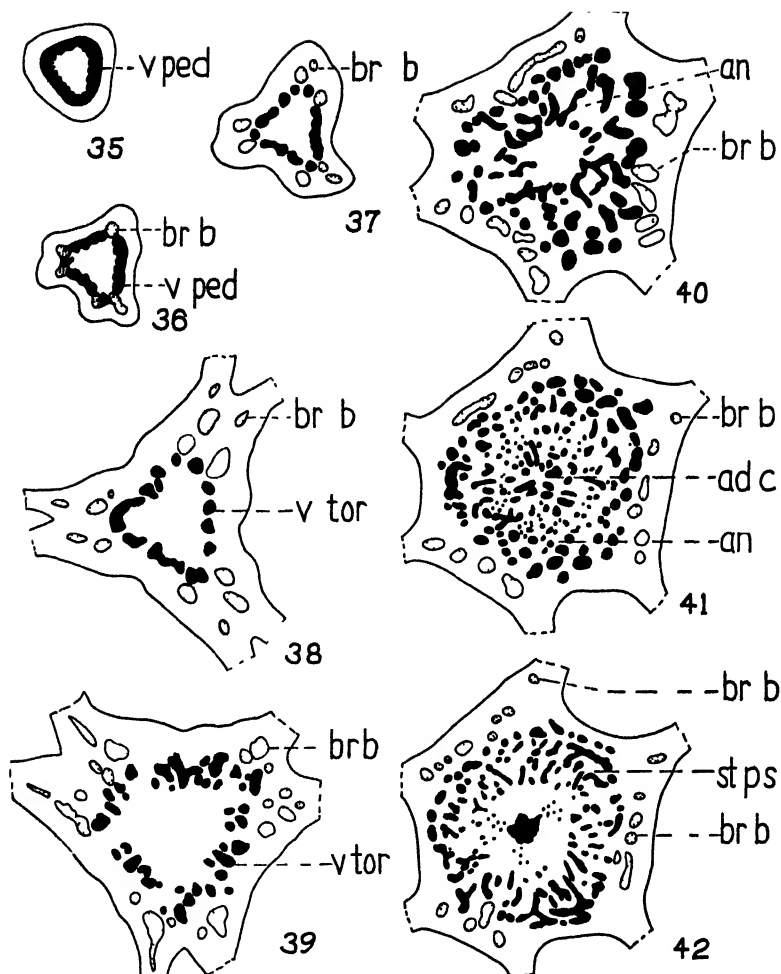
FIGS. 35–42.—Successive transections of flower bud showing vascular supply and various divergences at successively higher levels; Pima. Fig. 35, base of pedicel. Fig. 36, beginning of divergence of bract bundles; bract bundles stippled, rest of system solid black. Fig. 37, level of divergence of six bract bundles and breaking up of rest of cylinder. Fig. 38, further breaking up of bract bundles and widening out of remaining vascular bundles. Fig. 39, further outward divergence and anastomosing of bundles. Fig. 40, region of anastomosis or subcarpellary complex, with inward divergence of adaxial carpellary bundles and rest of system finely anastomosed. Fig. 41, adaxial carpel bundles at center of toral region; general anastomosis of bundles still evident. Fig. 42, adaxial group now a solid structure in the center: *v ped*, pedicel vascular supply; *br b*, bract bundles, also stippled; *v tor*, toral bundles; *an* region of anastomosis; *ad c*, adaxial carpellary system; *st p s*, stamen-petal-sepal region of anastomosis. Figs. 35–47 drawn to same scale.

Simultaneously with increase in size of the ovary, outgrowths are produced inside which, on growing inward and upward, develop into
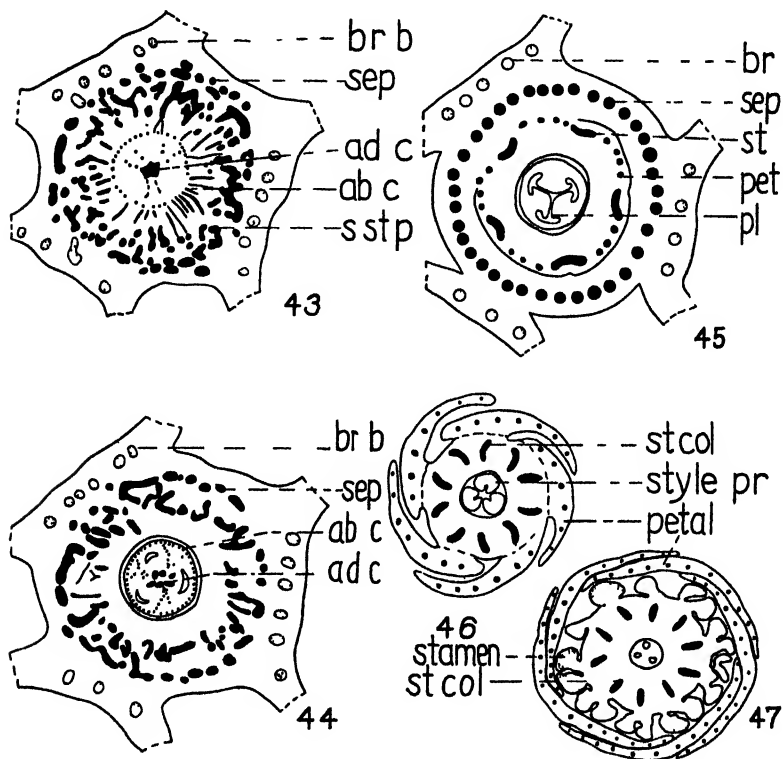
FIGS. 43–47.—Continuation of figs. 35–42; Pima. Fig. 43, region of anastomosis with inward divergence of many abaxial carpel bundles. Fig. 44, adaxial carpel bundles undiverged and two going to each carpel; stamen-petal group diverging inward from the still anastomosed sepal-petal-stamen complex which has continued undiverged from carpel base. Fig. 45, sepal ring completely separated from stamen-petal ring. Fig. 46, higher level on stamen-petal column. Fig. 47, region of complete divergence of all floral organs: *sep*, sepal bundle; *pet*, petal bundle; *s st p*, sepal-stamen-petal bundles; *st col*, staminal column bundle; *ab c*, abaxial carpellary bundles; *style pr*, style primordia; *pl*, placental ridges of septum.

the true septa on which the placental ridges are found (fig. 44). These septa arise separately and remain distinct for some time, finally coming together and touching in the center; but their edges never fuse completely except near the top. From the two vertical

edges of each septum the rounded placental ridges appear. The edges of these placental masses grow outward at an increasing angle toward the external carpel wall, finally becoming a flattened mass of meristematic tissue. On the placenta next arise the rounded ovule primordia. The development of the ovule has been traced in a previous paper (9) and will not be repeated here. As the ovules develop and the ovary enlarges, the edges of the placenta become closely appressed to the edges of adjacent septa.

The growing point of the axis can be seen in median longisections of young ovaries located in the center of the converging septa. It is possible that the height to which the end of the axis extends in the boll has something to do with incomplete opening of the boll at maturity. Externally the ovary increases greatly in diameter, bulging somewhat as it grows in height, and thus appears as a conical structure, bearing at the tip of each carpel a style.

The rapid increase in girth of the ovary portion comes about through the early origin and functioning of the false septum of each carpel, which in this case functions as a cambium. This layer later becomes a line of weakness in the ripe capsule, thus accounting for the loculicidal dehiscence of the fruit. The stylar projections early become folded laterally and remain folded as they grow in length. Their edges meet those of adjacent styles and complete coalescence of the tissues occurs, so that the locular cavities are completely closed at the top.

The tips of the styles continue to grow in length and several days before flowering project into the staminal tube. A stigmatic and pollen tube conducting region is early differentiated and occupies a median position with respect to the style as a whole, fitting like a wedge into the style and exposing only a lateral stigmatic surface (figs. 48–51). Stigmatic hairs develop only on the exposed lateral surface of the pollen tube conducting region (fig. 52). The styles of Pima and Sea Island are proportionately long and project beyond the anthers for perhaps half their length. Those of Mebane extend out slightly, if any, beyond the anthers. It is recognized that the distance which the stigmas project beyond the stamens has a great deal to do with the amount of natural crossing occurring in cotton.

ANATOMY.—The nodes on the main axis and branch axes have

three traces, which agrees with the general condition in the Mal-
vaceae as reported by SINNOTT (19).

The anatomy of the pedicel as in other plants (6) is essentially
that of a young stem. The primary vascular cylinder consists of
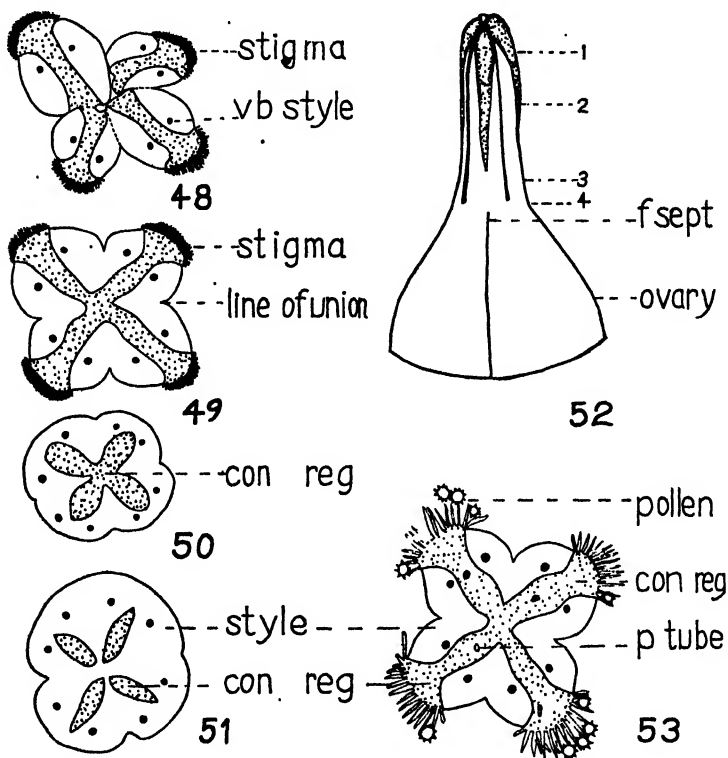
FIGS. 48–53.—Figs. 48–51, successive transections of stigma and style of Mebane.
Fig. 52, habit sketch of half-grown pistil; stigmatic region stippled: *1, 2, 3, 4*, levels of
figs. 48, 49, 50, and 51. Fig. 53, transection of upper part of styles of Upland cotton,
taken day of blooming; note pollen tubes in conducting region: *con reg*, pollen tube
conducting region; *p tube*, pollen tube; *f sept*, false septum.

twenty to thirty bundles arranged in a circle, each separated by rays.
At the place where the enlargement of the torus begins, the pedicel
becomes triangular and the vascular circle widens out at three defi-
nite points, also becoming triangular in outline (fig. 36). At a higher
level two bundles diverge outward at each corner of this triangle and
proceed to the outer and lowest edges of the bracts (fig. 37).

As the definitely enlarged toral part of the flower is reached other bundles diverge from the system and go to the bracts (fig. 39). At this level the entire vascular structure becomes somewhat funnel-shaped by the outward divergence of the whole system, a large pith region remaining.

At a higher level (fig. 40) several bundles from this system begin to diverge inward toward the center, while at the same time some of the bundles diverge toward the outside. These bundles, at first fairly distinct, soon anastomose to form a network of smaller bundles. The bundles comprising the inner portion of this region of anastomosis, or subcarpellary complex as it may be termed at this level, begin to diverge inward still more, as shown in figure 41. Most of the bract bundles have progressively diverged by the time this level is reached. Extensions of the inner diverging portion become the adaxial carpellary system or placental bundles. This anastomosing zone continues upward and diverges still farther inward. Figure 42 at a slightly higher level shows the large adaxial group, which is now one large bundle at the center of the torus, from which small bundles diverge and extend into the septa of the carpels. This main large adaxial bundle extends for only a short distance, however, before it is diverged into two distinct adaxial bundles to each carpel. From these adaxial bundles, as they continue upward in the boll, there extends a bundle to each of the ovules. The remnants of the adaxial system continue into the style.

The anastomosed sepal-petal-stamen bundles (fig. 42) continue upward for a short distance before the abaxial carpellary bundles are diverged (fig. 43). They extend inward almost horizontally and supply the outer carpel wall. There are several of these abaxial bundles supplying the outer wall of the boll, and their number varies with the number of carpels constituting the ovary. There are usually ten or more to each carpel. With the divergence of the abaxial bundles the carpellary system is complete. The false septum of each carpel consists of meristematic parenchyma and extends vertically in a median plane in each carpel. The anastomosed abaxial system of each carpel is thus separated by the false septum.

The central portion of each stylar segment consists of glandular tissue. Since the styles are fused together there appears a central re-

gion with three to five radiating areas (figs. 48–51). This region is continuous with the placenta of each carpel (5) and it is through this tissue that the pollen tubes pass to the micropyle of the ovule. This central pollen-tube conducting region is ensheathed by several layers of parenchyma in which the adaxial bundles are located. The unicellular stigmatic papillae are found on the surface wherever the pollen-tube conducting region is exposed (figs. 48–53).

Practically all the bundles supplying the bracts have diverged at the level of figure 44, although the bracts themselves are not distinct from the sepals until at a higher level. These bundles bifurcate until there are fifteen or more supplying each bract, the main veins extending to the tips of the teeth.

The anastomosed sepal-petal-stamen group of bundles continues undiverged for some distance above the separation from the carpels. At a higher level (fig. 45) the stamen-petal bundles diverge inward, leaving externally the ring of bundles which supplies the calyx cup. The sepal ring of bundles branch laterally somewhat, but chiefly continue on straight to the tips of the sepals.

The stamen-petal ring now consists of five large bundles that alternate with five groups of smaller ones, the petal bundles. The five large ones are the staminal column bundles. These five bundles, which can now definitely be called staminal column traces, extend farther inward and each divides into two equal sized bundles that come to lie opposed to each other, thus forming ten main radially arranged staminal column bundles which continue into the staminal column. The distance between two related bundles is increased by the development of a fleshy column, so that neither bundle now alternates with the petals but both appear opposite the petals. As these ten bundles ascend in the column, small bundles are given off from their outer edges to each stamen. These small stamen traces may branch one or more times, in keeping with branching of the stamens. One trace supplies an individual stamen. Ten principal bundles are constant for the staminal column of the three varieties studied. This number is also regularly found in *Malva* (20) and *Hibiscus* (11).

The petalline bundles, five groups of three or more each, soon branch and rebranch several times, and with considerable anasto-

mosing continue to the margins of the petals. The vein supply of the petals is similar in many respects to that of the calyx and bracts.

Summary

1. The ontogeny and development of the main axis, the fruiting branch, and the flower of three varieties of cotton are described. The vascular anatomy of the flower is also given.

2. The main stem consists of a single indeterminate primary axis from which leaves, stipules, and branches arise in acropetal succession from a terminal meristem.

3. The fruiting branch develops sympodially, and consists of a series of single internodal axes. As each axis is terminated by a flower, a new bud arises to continue the branch. This process is repeated each time a flower is formed.

4. From the flower primordium there arise three bract primordia, a sepal zone, a common petal-stamen zone from which stamens and petals are diverged almost simultaneously, followed by the carpel primordia in the order named.

5. The anomalous staminal column is developed from a basic number of five stamens, each proliferating to form a considerable number of stamen primordia on each of the five lobes of the column. Branching of stamen primordia further increases the number of stamens.

6. Three traces supply the nodes on the main axis and branch axes.

7. The vascular cylinder of the flower becomes anastomosed in the toral region. From this there is successively diverged: first, six bundles to the bracts, then the adaxial carpellary system, followed by the abaxial carpellary system at a higher level; next, five or more bundles supply each sepal, three or more bundles to each petal, and finally ten main bundles to the staminal column.

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LITERATURE CITED

1. BALLS, W. L., The sexuality of cotton. Yearbook Khed. Agr. Soc. Cairo, Egypt. 1905.

2. BEAL, J. M., A study of the heterotypic prophases in the microsporogenesis of cotton. La Cellule 38:247-268. 1928.

3. Cook, O. F., Morphology of cotton branches. U.S. Dept. Agr. Bur. Plant Ind. Circl. 109. 1913.

4. Cook, O. F., and Meade, R. M., Arrangement of parts in the cotton plant. U.S. Dept. Agr. Bur. Plant Ind. Bull. 222. 1911.

5. Coulter, J. M., and Chamberlain, C. J., Morphology of angiosperms. University of Chicago Press, Chicago. 1915.

6. Eames, A. J., and MacDaniels, L. H., An introduction to plant anatomy. McGraw Hill, New York. 1925.

7. Farr, W. K., Origin and early stages of elongation. Contrib. Boyce Thompson Inst. 3:441–458. 1931.

8. Goebel, K., Organography of plants. Eng. Transl. 1905.

9. Gore, U. R., The development of the female gametophyte and embryo in cotton. Amer. Jour. Bot. 19:795–807. 1932.

10. Gray, A., Structural botany. 6th ed. American Book Co., New York. 1879.

11. Henslow, G., On the vascular systems of floral organs, and their importance in the interpretation of the morphology of flowers. Jour. Linn. Soc. Bot. London 28:151–197. 1891.

12. Kearney, T. H., Genetics of cotton. Jour. Heredity 21:325–336; 375–384; 409–415. 1930.

13. King, C. J., Development of axillary buds on fruiting branches of Pima and Upland cotton. Jour. Agr. Res. 41:697–714. 1930.

14. Leake, H. M., Studies on Indian cotton. Jour. Genetics 1:205–272. 1911.

15. Luyten, Ida, De Periodiciteit van de Knopontwikkeling bij den Pruim. Mededeelingen van de Landbouwhoogeschool. Wageningen Deel XVIII. Lab. v. Plantenphysiologie 4:103–148. 1921.

16. McClelland, C. K., and Neeley, J. W., The order, rate, and regularity of blooming in the cotton plant. Jour. Agr. Res. 42:751–763. 1931.

17. McLachlan, A., The branching habits of Egyptian cotton. U.S. Dept. Agr. Bur. Plant Ind. Bull. 249. 1912.

18. Robbins, W. W., Botany of crop plants. 3rd ed. Blakistons, Philadelphia. 1933.

19. Sinnott, E. W., The anatomy of the node as an aid in the classification of angiosperms. Amer. Jour. Bot. 1:303–322. 1914.

20. Smith, A. L., The floral development of *Malva rotundifolia*. Unpublished thesis, University of Chicago. 1931.

21. Spieth, Alda M., Anatomy of the transition region in *Gossypium*. Bot. Gaz. 95:338–347. 1933.

22. Zaitzev, G. S., A contribution to the classification of the genus *Gossypium* L. Trans. Turkestan Plant Breeding Station. Tashkent. No. 12. 1928.

HISTOLOGICAL VARIATIONS IN COSMOS IN RELATION TO PHOTOPERIODISM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 468

ORLIN BIDDULPH

(WITH TWENTY-NINE FIGURES)

Introduction

GARNER and ALLARD (8, 9, 10) have described the response of *Cosmos* when subjected to various photoperiodic treatments. Their investigations show that *C. sulphureus* Cav. is a short day plant growing vegetatively in long days and flowering when the daily light period is shortened to ten hours. It can be grown to a height of 15 feet or more or made to flower in 50 days, depending on the length of the daily light period. The age and size of the plants make no difference in the speed with which they react to the change in the length of the daily light period. By the use of an electric light to prolong the light period of the normal day, it was found that an intensity of 4-foot candles was effective in producing vegetative growth in short day plants and reproductive activity in long day plants.

When short day plants which have been given a 10-hour day for 15 days are transferred to conditions of a long day, only a few of the blossom buds already initiated finally open, the others being permanently suppressed. Vegetative shoots from the axillary buds on the axis below the apical flower bud soon begin to elongate and the latter is apparently deprived of the necessary nutritive materials for development.

GARNER, ALLARD, and BACON (11) have applied the concept of the carbohydrate-nitrogen relation as a factor in flowering and reproduction (16) to photoperiodic response, and have found that a change in soluble carbohydrate accompanies a change in the length of the daily light period. In *Cosmos* there is an increase in reducing sugars in the apex of the stem within 48 hours after the transfer of the plant from long to short days.

NIGHTINGALE (19) has stated that in general plants high in carbohydrates have relatively much of their assimilated nitrogen as protein, although the percentage of protein may be low, and has suggested that the duration of the light period is a factor in the utilization of carbohydrate for the conversion of inorganic nitrogen into protein form. A shift in the day length brings about a reworking of the metabolized products and an establishment of a new equilibrium between soluble and insoluble forms. Vegetative activity prevails when a high percentage of the metabolized nitrogen is present in a relatively soluble form, whereas flowering and reproduction are accompanied by an accumulation of carbohydrate and a decrease in soluble forms of nitrogen.

ADAMS (1, 2) has shown that plants exposed to a diminished light supply have a more rapid growth rate at first but ultimately the ones receiving the most light make the most growth. GILBERT (12, 13), in his work on the photoperiodic responses of *Xanthium*, found that under both high and low temperature conditions the ratios of total carbohydrate to total nitrogen and soluble carbohydrate to soluble nitrogen were distinctly ascending as flower primordia were formed.

ECKERSON (6) has found in Biloxi soy beans grown in an 8-hour day and a 16-hour night a decrease in nitrate reducing power of more than 95 per cent, and KNOTT (15) has found that the proper day length for the induction of flowering in *Cosmos* reduced the catalase activity in the stem tip to an extent of 109 per cent in 20 days. The length of the daily light period may also control the relative amounts of carotenoid pigments (18). CHIBNALL (5) found that at the time of flower bud formation asparagine is the most prominent product of the protein metabolism.

These investigations indicate that there are definite physiological changes accompanying flowering and reproduction. These changes have been induced entirely by the length of the daily light period as the external nutrient supply has remained constant. It is possible then to induce flowering entirely apart from any nutrient treatment. Flowering so induced is accompanied by a definite chemical change in the region of the apical primordium. The present study repre-

sents an attempt to determine more precisely the exact anatomical response of the primordium to the day length and the progressive chemical changes accompanying or preceding such anatomical change.

Methods

Plants of *Cosmos sulphureus* Cav. var. Klondike were grown from seed in rich soil, in boxes 1×1×1 feet. They were kept under long day conditions by the use of 1000 watt Mazda lamps supplying an intensity of approximately 75 foot candles at the tops of the plants. Plants were grown under both 15- and 16-hour days, the lamps supplementing the particular number of daylight hours prevailing at the time of the experiment. Ordinary greenhouse temperatures prevailed. During the short day treatment the plants were kept at the same temperature as the long day controls. In the series run in May the night temperature varied between 65° and 70° F. while the day temperature rose to 90° or 100° F. for a short period during the afternoon. In the series grown in January the temperature remained nearly constant at 76° during the day and 65° F. at night.

Two methods of artificially producing short days were used. In May, when the temperatures were high at night, a screen of double thickness black sateen cloth was placed over the framework surrounding the plants at 4:30 P.M. and removed at 8:30 A.M., giving an 8-hour day. In winter the boxes were placed on a truck and moved into a dark chamber at 4:30 P.M. and out at 9:00 A.M., giving a 7½-hour day.

Only relatively short periods of short day treatment are necessary to induce flowering in *Cosmos*. Accordingly separate groups of plants were given 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14 consecutive short days respectively, and then exposed to long day treatment. Plants of each group were then examined periodically to determine the least number of short days necessary to induce flowering. The retarding effect of the subsequent long day treatment was determined by comparison with plants under short day treatment. A dissecting microscope proved the most useful means for determining the morphological response of the apical primordium. Material was also imbedded and prepared according to the usual paraffin method.

Microchemical as well as macrochemical analyses were made at various stages indicated as being significant by the detailed anatomical examination.

Investigation

MICROCHEMICAL METHODS

Periodic examinations were made in order to follow the progress of the chemical changes occurring after the short day treatment was begun. Apical primordia of primary or lateral axes of purely vegetative long day plants were used in each case as a standard of comparison. The primordium itself and only the very closely associated stem tissue were used in the stem tip sample. The base of the stem was also examined to determine whether or not there was a change in this region accompanying the changes in the primordium. Comparable sections through stem tips of the long and short day plants were made by means of a sliding microtome in which both stems had been fastened by clamping them side by side in cotton, so that both were sectioned by the same stroke of the knife.

The microchemical tests found to be the most useful were:

GLUTATHIONE.—Based on the nitroprusside reaction of HOPKINS, modified by FINK (7), this was used as follows: Heat sections in 10 per cent acetic acid to boiling and remove to three drops of saturated ammonium sulphate. Add two drops of 5 per cent sodium nitroprusside $(Na_2Fe(CN)_5 \cdot NO \cdot 2H_2O)$. Allow to stand for 5 minutes. Add an excess of concentrated ammonium hydroxide (two or three drops). A pink to permanganate color indicates the presence of glutathione. The depth and duration of the color have been shown to indicate the amount present.

ARGININE.—Based on the SAKAGUCHI (21) reaction which is specific for the free-guanidine group and therefore for arginine, this was used as follows: Place sections in one drop of 15 per cent NaOH. Add two drops of 0.15 per cent alpha naphthol. Add three or more drops of 0.5 per cent sodium hypochlorite. Red color on standing indicates arginine. It may be used quantitatively.

HISTIDINE AND TYROSIN.—Based on Ehrlich's diazo reaction, this was used as follows: One drop of 0.5 per cent solution of sulphanilic acid in 2 per cent hydrochloric acid. Allow to react with one drop

of 0.5 per cent sodium nitrite for a minute or two. Add section to
this and make the mixture alkaline with 10 per cent sodium carbon-
ate. The presence of histidine is indicated by a deep red color.
Tyrosin gives more of an orange tinge. The reaction can be em-
ployed quantitatively.

CONDENSATION REACTIONS WITH TRYPTOPHAN.—Treat sections
with a 5 per cent solution of formalin for 15 minutes, then add strong
hydrochloric acid to the mixture. A trace of an oxidizing agent such
as ferric salts or nitrites speeds the reaction.

TYROSIN.—Based on the use of Millon's reagent as modified by
BENSLEY (3).

TOTAL PROTEIN.—Based on various reactions; with I_2KI, ninhy-
drine, Millon's reagent, Sakaguchi reaction, etc.

FRUCTOSE.—Based on the reduction of Fehling's solution in the
cold.

TOTAL REDUCING SUGAR.—Based on the reduction of Fehling's
solution when heated.

Inulin, which is a storage product of *Cosmos*, seems to have a
peculiar effect on the reduction of Fehling's solution by the reducing
sugars, causing the formation of an amorphous copper complex
which contains some crystals of Cu_2O in its matrix. This difficulty
was minimized by diluting the Fehling's solution (one part of mixed
Fehling's solution to three parts of water). Crystals of Cu_2O are
also larger if this concentration is used.

MACROCHEMICAL ANALYSES

After the critical number of short days necessary to induce flower-
ing was determined and the subsequent response of the primordia
known, it was possible to bring the plants to any desired anatomical
stage for macrochemical analysis. A period of ten short days was
selected as an appropriate treatment of material to show the chem-
ical differences between plants which had received the necessary
stimulus to flower and those which would remain strictly vegetative
under long day conditions.

The average height of the plants used for analyses was 12 inches.
The stems were cut just above the ground and divided into two
parts, the stem tip sample including the first inch of stem tip, and

the stem base sample including the remainder of the stem. Nitrogen fractionations and carbohydrate determinations were made on each sample.

ANATOMICAL OBSERVATIONS

All plants do not react to short day treatment with exactly the same speed. Those which react the fastest may precede the slower

FIGS. 1–9.—Figs. 1–3, median sections through primordium of plants which have received seven 8-hour days followed by seven 16-hour days; figs. 4–6, seven 8-hour days followed by fourteen 16-hour days; figs. 7–9, seven 8-hour days followed by eighteen 16-hour days.

plants in the formation of flower buds by two or three days. Seven short days was the least number which produced an anatomical response, regardless of whether a 7½-hour or an 8-hour daily light period was used. The plants which were exposed to an 8-hour daily light period in May responded 100 per cent within 9 days, whereas ten days were required for 100 per cent response under a 7½-hour

daily light period in January. Figures 1–9 show the variations in the response of *Cosmos* primordia to seven 8-hour days in May.

It was thought that by reducing the length of the period of short day treatment to the absolute minimum necessary for the differentiation of flower primordia and then exposing the plants to the conditions of the long day, some expression of interphases (or "vegetative
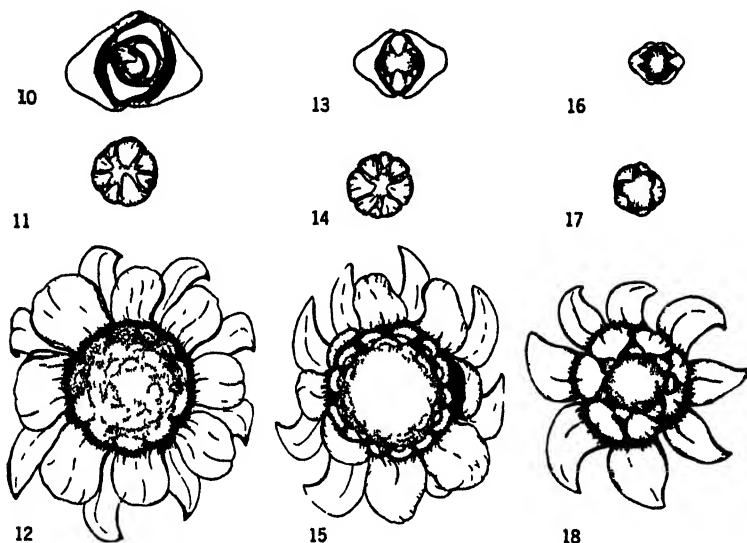
FIGS. 10–18. - Fig 10, view of primordium of a plant which has changed from an opposite to a spiral leaf arrangement; fig 11, apical primordium from a plant which has received fourteen 7½-hour days; fig 12, twenty-one 7½-hour days (the two sets of bracts are much further developed than the individual flower primordia); fig 13, nine 7½-hour days (no bracts formed at this time); fig. 14, nine 7½-hour days followed by seven 15-hour days (first row of bracts just developing); fig 15, nine 7½-hour days followed by fourteen 15-hour days; fig. 16, eight 7½-hour days; fig. 17, eight 7½-hour days followed by seven 15-hour days; fig. 18, eight 7½-hour days followed by fourteen 15-hour days.

flowers") would appear. In the group of plants given short day treatment in May, however, little tendency toward interphases appeared, save for the change from opposite to spiral leaf arrangement in 18 per cent of the plants (fig. 10).

The fact that all plants in the process of differentiating flower primordia gave strong evidence, after three weeks of subsequent long day treatment, of developing normal flowers, indicated that the light intensity or the daily light period might be further reduced and

a still closer approach to the minimum light requirement for flowering found. The same type of experiment was therefore made in January when the light intensity in Chicago is much lower. The daily light period was reduced to $7\frac{1}{2}$ hours. Groups of plants were exposed to 6, 7, 8, 9, and 10 short days respectively, after which they

TABLE I

RESPONSE OF COSMOS PLANTS TO DAILY LIGHT PERIOD OF 8 HOURS
IN MAY, EXPRESSED IN PERCENTAGE OF NUMBER
OF PLANTS USED

No. of SHORT DAYS	REMAINING VEGETATIVE	CHANGING TO SPIRAL	FORMING FLOWER PRIMORDIA	No. of PLANTS
6............	100	....	....	9
7............	41	18	41	39
9............	....	....	100	6

TABLE II

RESPONSE OF COSMOS PLANTS TO DAILY LIGHT PERIOD OF $7\frac{1}{2}$ HOURS
IN JANUARY, EXPRESSED IN PERCENTAGE OF
NUMBER OF PLANTS USED

No. of SHORT DAYS	REMAINING VEGETATIVE	CHANGING TO SPIRAL	FORMING FLOWER PRIMORDIA	No. of PLANTS
6..........	100	....	... .	10
7..........	67	20	13	15
8........	40	11	49	26
9..........	20	27	53	15
10..........	....	....	100	10

were removed to conditions of the long day. Electric lights (1000 watt Mazda lamps, giving approximately 75 foot-candles intensity at the top of the plants) were used to supplement the normal day length, giving a 15-hour day. The results obtained are shown in tables I and II.

The differences in the rates of development of flowers by plants which had received 7, 8, and 9 short days are shown in figures 10–18. The retarding effect on floral development produced by long days following the short day treatment may be seen by comparing plants

that were shifted to long day conditions with those that were maintained continuously under short day illumination (figs. 11, 12).

A third group of plants which had attained a height of 12–16 inches was divided into three lots. The first lot received eight $7\frac{1}{2}$-hour days, the second, nine $7\frac{1}{2}$-hour days, and the third, ten $7\frac{1}{2}$-hour days, each being immediately placed under conditions of the long day after the treatment. These plants were allowed to grow in the long day conditions (15 hours of light per day) for three months. At the end of this period a very decided difference in the floral development was found.

The normal *Cosmos* flower has two rows of involucral bracts, usually eight in each row. The outer set is foliaceous and spreading while the inner set is broader and nearly membranous. The ray flowers, usually eight in number, appear in the axils of the inner set of involucral bracts. Each disk flower is borne in the axil of a bractlet on the determinate composite head. The bracts ordinarily remain shorter than the disk flowers and are membranous in nature.

The first flowers to open in the foregoing group were those which had received ten short days. Two months of long days were required before the first flowers opened. The control plants, which were kept under continuous short day conditions, began to blossom in six weeks. The following differences in the flowers were noticed (figs. 26–29):

Continuous short day treatment until flowering ($7\frac{1}{2}$-hour days)
    Length of first row of bracts............. 5 mm. (fig. 28)
    Length of second row of bracts......... . 9 mm.
    No. of ray flowers....8; length.......... 20 mm. (fig. 27)
Ten short days followed by two months of long days ($7\frac{1}{2}$- and 15-hour days)
    Length of first row of bracts............. 16 mm. (fig. 29)
    Length of second row of bracts.......... 12 mm.
    No. of ray flowers.. .14; length......... 20 mm. (fig. 26)

Figures 19 to 25 show the types of variations from normal flowers which were found. There are four important points concerned with their development. First, the primordium initiates a flower and becomes determinate, but owing to the influence of the long days the bracts and bractlets elongate and become foliaceous and the flowers
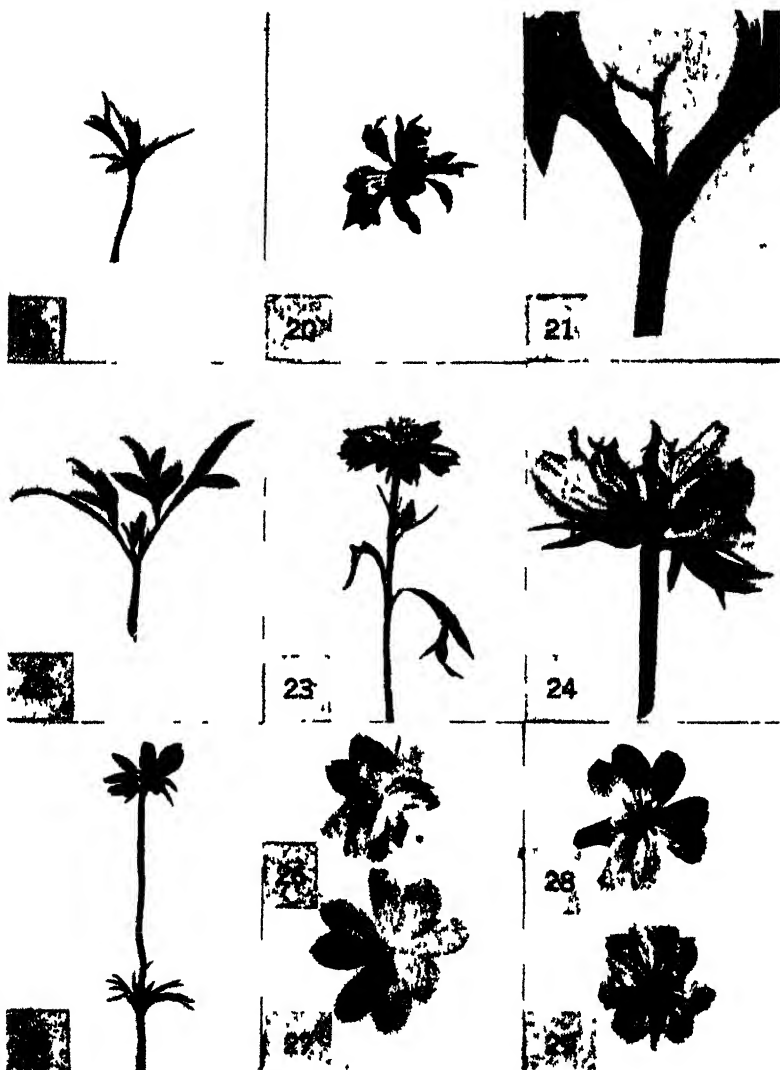
FIGS 19–29 —Figs. 19, 20, apex of stems from plants which have received eight 7½-hour days followed by sixty 15-hour days (bracts have become foliaceous and flowers are suppressed); fig. 21, eight 7½-hour days followed by sixty 15-hour days (flower bud suppressed); figs. 22–24, developing flowers from plants which have received nine 7½-hour days followed by sixty-five 15-hour days (division of flower primordium shown); fig 25, nine 7½-hour days followed by sixty-five 15-hour days (abnormal elongation of stem between bracts and flowers shown); figs. 26, 29, front and rear views of flower from a plant which has received ten 7½-hour days followed by sixty 15-hour days; figs. 27, 28, front and rear views of flower from a plant which has received continuous treatment under 7½-hour daily light period.

fail to develop beyond mere appendages in their axils (figs. 19, 20). Second, if the tendency to flower is a little stronger the flower head may develop to a diameter of about 3 mm. and then become permanently suppressed. The axillary buds immediately below become active vegetatively and develop into new shoots which assume apical dominance (fig. 21). Third, if the tendency to flower is still stronger the flower head may develop and produce a mature flower but there is a marked tendency for the primordium to divide, resulting in two flower heads. The division may result in two flower stalks of equal length, or the division may not result in two flower stalks but in the appearance of two flower heads on one stalk (figs. 23, 24). Fourth, when there is considerable retardation of floral development by the long days, an internode of considerable length may develop between the involucral bracts and the bractlets of the floral head (fig. 25); or the elongation of the stem in the region of the involucral bracts may result in their separation longitudinally, leaving them spirally arranged over a distance of 1-2 cm. of the stem immediately below the flower head.

MICROCHEMICAL RESULTS

Vigorously vegetative plants were taken from a 15-hour day and given a $7\frac{1}{2}$-hour day. Thereafter examinations were made daily at 9:00 A.M. and 7:00 P.M. for a period of 14 days. Long day strictly vegetative plants were used as a standard of comparison at each examination.

From the morning and evening examination of the primordia a diurnal fluctuation in the glutathione content was discovered. It was found that in the morning the primordia of the short day plants contained a considerably greater quantity of glutathione than those of the plants which had received a long day. After both plants had been exposed to a period of light lasting until evening, however, the glutathione content of the primordia was approximately the same. This nightly fluctuation of glutathione in the short day plants continued until about the seventh day, when it became and remained permanently greater than in the vegetative plants. This observation was extended to the fourteenth day, at which time the flower primordium is well developed.

CARBOHYDRATES.—A marked increase in reducing sugars appeared in the stem tips of the short day plants by the fourth day of treatment. From that time on to the fourteenth day, when observations were discontinued, there was a progressive increase in the amount present. The accumulation of reducing sugar appeared before there was any marked increase in the protein content of the stem tip.

NITROGENOUS FRACTIONS.—There was no marked increase in the total amount of organic nitrogenous material in the stem tips of the short day plants during the fourteen days of observation. The relative amount of soluble and insoluble organic nitrogenous products, however, changed markedly. In the stem tips of the plants which had received short day treatment there was an accumulation of proteinaceous material beginning about the fifth or sixth day and becoming marked by the seventh day, from which time it continued to increase. The protoplasm of the cells increased in density.

As the reactions employed for the detection of amino acids are given by both the free amino acids and proteins, there was some difficulty in the determination of the relative amounts of protein and soluble organic nitrogen. This difficulty was best overcome by denaturing the proteins before the color reactions were employed. The proteinaceous material then gave a much better test with Millon's reagent and ninhydrine reactions than undenatured material. With reasonable care in the preparation of the sections, and the comparison of one section with another, there was little difficulty in demonstrating the greater accumulation of complex protein material in the stem tips of the short day plants than in the vegetative tips of long day plants.

MACROCHEMICAL RESULTS

The tenth day after the beginning of the short day treatment was selected as the best time for the collection of samples for analysis, for by that time all plants would have received the necessary stimulus to flower yet there would have been no marked anatomical change. Purely vegetative long day plants were used as the controls.

Carbohydrate analyses were made by mincing the fresh tissue and extracting with hot 95 per cent alcohol. The soluble reducing sugars were determined therefrom by the Bertrand volumetric method.

The insoluble residue from the extraction was boiled in 2.5 per cent hydrochloric acid for three hours and the hydrolyzable carbohydrates determined by the same method. Sucrose was not determined.

Fresh tissue only was used for nitrogenous fractionations. Extractions were made with water according to the methods of CHIB-

TABLE III

ANALYSIS OF PLANTS HAVING RECEIVED 10 SHORT DAYS, AND LONG DAY VEGETATIVE PLANTS, EXPRESSED IN PERCENTAGE OF GREEN MATTER

	SHORT DAY STEM TIPS	LONG DAY STEM TIPS	PERCENTAGE DIFFERENCE	SHORT DAY STEM BASES	LONG DAY STEM BASES	PERCENTAGE DIFFERENCE	SHORT DAY WHOLE STEM	LONG DAY WHOLE STEM	PERCENTAGE DIFFERENCE	PERCENTAGE VARIATION OF SAMPLES
NH_3.....	0.0032	.0128	75.0	0.0047	.0095	50.5	0.0079	.0223	64.6	15
Amide......	0.0378	.0284	24.7	.0140	.0182	23.1	.0518	.0466	10.0	15
NO_3.........	.0061	.0061	00.0	.0054	.0090	40.0	.0115	.0151	23.8	15
Soluble organic N.....	.1247	.1328	6.1	.1497	.1366	8.7	0.2726	.2694	1.2	2
Protein N....	.2315	.2155	6.9	.0820	.1080	24.1	.3125	.3235	3.4	2
Total organic N.........	.3562	.3483	2.2	.2299	.2446	6.0	.5861	.5929	1.1	2
Hydrolyzable carbohydrate....	1.515	1.515	0.0	1.380	1.650	16.4	2.895	3.165	8.5	2
Reducing carbohydrate	0.5360	0.4640	13.4	0.4800	.5360	10.4	1.016	1.000	1.6	2
Dry weight	8.980	9.310	3.5	8.000	9.550	16.2	8.490	9.430	10.0	2

NALL (4) and determinations were made according to the usual methods (20). All samples were made in duplicate and checks were run on each sample. All differences greater than 2 per cent are significant except for ammonium, nitrate, and amide nitrogen. Here the variations between the several extractions differ by 15 per cent. The difficulties involved in sampling and extracting such small aliquots make the variability of results high.

Discussion

Cosmos is affected so strongly by a short daily light period that it will flower and set seed after being exposed to only ten $7\frac{1}{2}$-hour

days. When flower primordia are initiated by a fewer number of short days the tendency to flower has not always been so definitely established that it may not be reversed by subsequent treatment with long days, producing "vegetative flowers." The short day treatment obviously has some marked effects on the chemical processes associated with flowering. The chemical changes accompanying the anatomical change agree closely with other observations concerning flowering and reproduction, especially in regard to the carbohydrate-nitrogen relation (16).

The composition of the whole plant does not change markedly, except that there is a decrease in dry weight of 10 per cent during the ten short days (17). This can be accounted for mainly by the decrease in polysaccharides of 8.5 per cent and a slight decrease in protein. As the amount of materials manufactured during the shortened days is markedly less, it seems important to consider the states of the metabolized materials as they are related to the daily light period. Under the shortened light exposure there is a marked tendency toward the concentration of soluble carbohydrate and complex protein in the stem tip at the expense of the polysaccharides and proteins in the base of the stem. There are differences in asparagine and ammonia and undoubtedly in many other cellular constituents, but a more complete study of the composition of the proteins is needed in order to give them meaning. The important point is that it requires approximately seven short days for the stem tip to acquire a definite and marked accumulation of reducing sugars, and a change in the state of the proteins toward a much more complex and difficultly soluble form. Any treatment involving fewer short days than is necessary to bring about this change is without effect in inducing flowering, but a few added days of treatment, once the accumulation has begun, giving time enough for the accentuation of the changes, insured 100 per cent conversion of the vegetative primordia to the flowering condition.

The initiation of a flower primordium is associated with a definite metabolic state of the stem tip. The changes in the metabolism of the vegetative plant which result in flowering due to photoperiodic treatment include a probable proteolysis of the proteinaceous material and the hydrolysis of polysaccharides in the base of the stem

and their removal to the stem tip. The photoperiod influences (1) the amount of material which the plant is able to conserve, (2) the use to which it will be put, and (3), the quality of the proteins and carbohydrates in the various regions.

The amount and kind of carbohydrate and protein as determined in these experiments, and the diurnal fluctuation in the amount of glutathione, seem to be factors intimately connected with the photoperiodic treatment which has a definite correlation with the morphological change of the primordium from vegetative to reproductive. Any conclusions as to the meaning of the fluctuation of glutathione content with the change in the daily light period cannot be made until more is known concerning the function of glutathione. In the light of certain recent findings by HAMMETT (14), it might seem that glutathione is a factor which modifies the metabolism of proteins. At any rate it seems evident that both carbohydrates and proteins are influenced by the photoperiod, but whether it is directly or indirectly, through the action of some intermediate compound, has not been determined.

Summary

1. The plants of *Cosmos sulphureus* Cav. var. Klondike reacting most readily to short day treatment required seven short days for the initiation of a flower primordium. The slowest plants required ten days. When the absolute minimum treatment necessary to induce flowering was given, followed by long days, there was a marked tendency toward the production of interphases between normal flowers and vegetative shoots.

2. The change of the primordium from foliar to floral was accompanied by a marked accumulation of carbohydrate and protein in the stem tip. There is a probable proteolysis and a decrease in protein in the base of the stem at the time of the conversion of the primordium to the flowering state. There is also hydrolysis of carbohydrates in the base of the stem and their removal to the stem tip at the same time.

3. The shortened light period caused a decrease of 1 per cent per day of the total materials stored.

4. There was a diurnal fluctuation of glutathione in the stem tip during the first seven days of short day treatment, but from the

time of the actual anatomical shift of the primordium from vegetative to flowering the total glutathione content remained permanently higher.

5. Asparagine was somewhat higher and ammonium somewhat lower in the stem tip at the time of flower bud formation.

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LITERATURE CITED

1. ADAMS, J., The effect on certain plants of altering the daily period of light. Ann. Bot. **37**:75–94. 1923.
2. ———, Duration of light and growth. Ann. Bot. **38**:509–523. 1924.
3. BENSLEY, R. R., and GERSH, I., Studies on cell structure by the freezing-drying method. Anat. Rec. **57**:228. 1925.
4. CHIBNALL, A. C., Investigations on the nitrogenous metabolism of the higher plants. Part III. The effect of low-temperature drying on the distribution of nitrogen in the leaves of the runner bean. Biochem. Jour. **16**:599–611. 1922.
5. ———, Investigations on the nitrogenous metabolism of the higher plants. Part VI. The rôle of asparagine in the metabolism of the mature plant. Biochem. Jour. **18**:395–405. 1924.
6. ECKERSON, SOPHIA H., Conditions affecting nitrate reduction by plants. Contr. Boyce Thompson Inst. **4**:119–130. 1932.
7. FINK, D. E., A micro method for estimating the relative distribution of glutathione in insects. Science **65**:143–145. 1927.
8. GARNER, W. W., and ALLARD, H. A., Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agr. Res. **18**:553–606. 1920.
9. ———, Further studies in photoperiodism, the response of the plant to relative length of day and night. Jour. Agr. Res. **23**:871–920. 1923.
10. ———, Localization of the response in plants to relative length of day and night. Jour. Agr. Res. **31**:555–556. 1925.
11. GARNER, W. W., BACON, C. W., and ALLARD, H. A., Photoperiodism in relation to hydrogen-ion concentration of the cell sap and the carbohydrate content of the plant. Jour. Agr. Res. **27**:119–156. 1924.
12. GILBERT, B. E., The response of certain photoperiodic plants to differing temperature and humidity conditions. Ann. Bot. **40**:315–320. 1926.
13. ———, Interrelation of relative day length and temperature. BOT. GAZ. **81**:1–24. 1926.
14. HAMMETT, F. S., A possible explanation of the function of glutathione in developmental growth. Science **79**:457. 1934.

15. KNOTT, J. E., Further localization of the response in plant tissue to rela-
tive length of day and night. Proc. Amer. Soc. Hort. Sci. 23:67–70. 1926.

16. KRAUS, E. J., and KRAYBILL, H. R., Vegetation and reproduction with
special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149. 1918.

17. LUBIMENKO, V. N., and SZEGLOVA, O. A., L'adaption photopériodique des
plants. Rev. Gén. Bot. 40:675–689. 1928.

18. MURNEEK, A. E., Relation of carotinoid pigments to sexual reproduction in
plants. Science 79:528. 1934.

19. NIGHTINGALE, G. T., The chemical composition of plants in relation to
photoperiodic changes. Univ. Wisconsin Agr. Exp. Sta. Bull. 74 1927.

20. NIGHTINGALE, G. T., SCHERMERHORN, L. G., and ROBBINS, W. R., Some
effects of potassium deficiency on the histological structure and nitrogenous
and carbohydrate constituents of plants. New Jersey Agr. Exp. Sta. Bull.
499. 1930.

21. SAKAGUCHI, S., Über eine neue Farbenreakion von Protein und Arginine.
Jour. Biochem. Tokyo 5:25–31. 1925.

PENNSYLVANIAN FLORA OF ILLINOIS AS REVEALED IN COAL BALLS. II.

ROY GRAHAM

(WITH TWENTY-FOUR FIGURES)

The following descriptions are based on coal ball material from the Calhoun coal mine, Richland County, Illinois, horizon McLeansboro (Upper Conemaugh). The specimens which form the basis of these descriptions are in the writer's collection, but duplicate sections of most of the material are in the collections of the botany departments of the universities of Cambridge and Chicago.
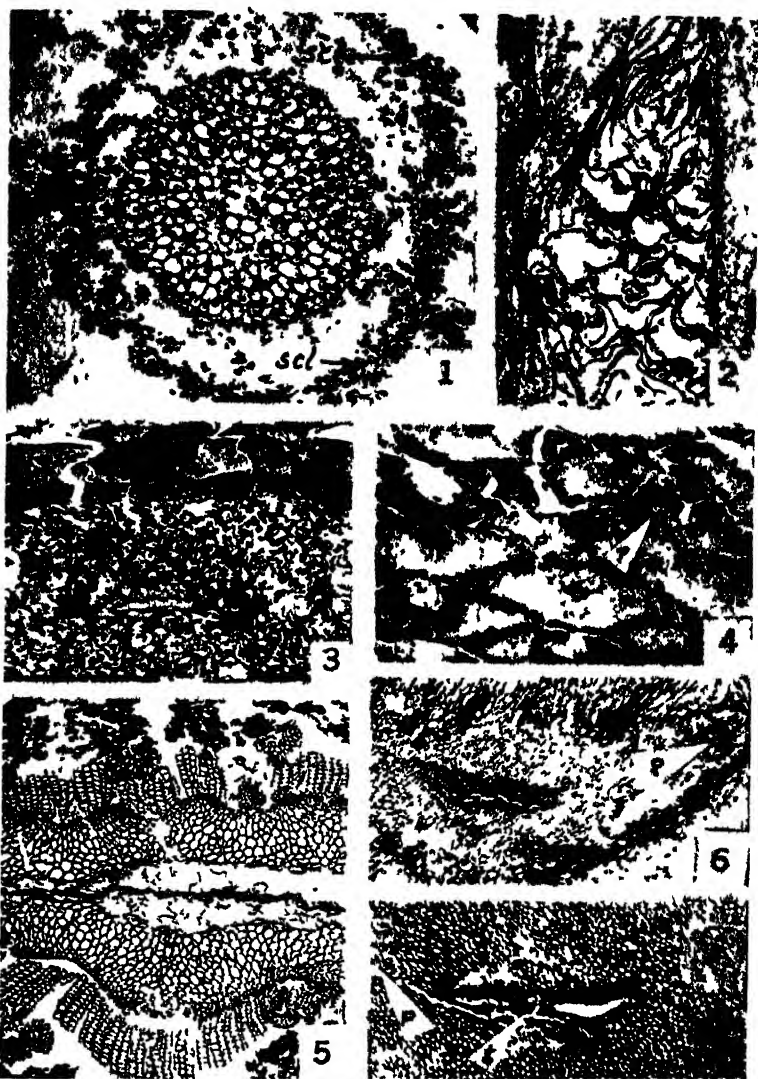
Lepidodendron cf. *L. selaginoides* Sternb.

A number of small branches and a number of closely associated leaves were found in two coal balls. The largest branch measures 1.2 cm. in diameter and has a stele 0.2 cm. in diameter. The smooth outline of the corona and the presence of a few tracheids in the pith suggest a comparison with *L. selaginoides* (9), or with *L. hickii* Wats. (14). There are a greater number of tracheids in the pith of *L. selaginoides* than in *L. hickii*, but in these small specimens the pith area is too limited to make certain identification on the basis of this character. The presence of a ring of sclerenchyma (*scl*, fig. 1) in the inner cortex suggests identification with *L. selaginoides*.

Lepidostrobus sp.

A number of small lycopodiaceous strobili occur in one coal ball. The strobili are of two kinds, one containing megaspores and the other microspores. Detailed description is impossible on account of poor preservation.

Several longitudinal tangential sections of the tip of one strobilus give the best clue to the structure. The most nearly radial section (fig. 2) just grazed the cortex of the central axis. The portion preserved measured 15 mm. long by 3.5 mm. in diameter. The sporophylls stand out almost at right angles to the axis; they are very thick at the base and are joined to the axis by a short stout attach-

Figs 1 7 — *Lepidodendron* cf *L selaginoides*, transverse section of stele and en circling zone of sclerenchyma (*scl*), ×20 Fig 2, *Lepidostrobus* sp , longitudinal section of tip of strobilus, ×8 Figs 3–7, *Sigillaria approximata* 3, transverse section of stem ×2, 4, longitudinal tangential section through leaf bases and vertical of peduncles (*p*) ×2, 5, transverse section of portion of stele ×20, 6, longitudinal tangential section in region of secondary cortex showing leaf trace (*t*) surrounded by parenchyma which is continuous with parichnos (*p*) ×20, 7, a more superficial section of the same, exterior to the secondary cortex (*t*, leaf trace, *p*, parichnos), ×20

ment; there is no definite pedicel. The sporophyll is broad and winged, and its leaflike lamina does not have a downward projection. The sporangia are only slightly elongated in the radial direction and are attached to the upper surface of the sporophyll by a pad of transfusion tissue. The sporangium wall consists of a single prismatic layer. Only fragments of thick walled megaspores remain.

Transverse sections measuring about 20 by 4 mm. are too badly crushed and disorganized to show the shape and arrangement of the sporophylls. The axial stele, 0.38 mm. in diameter, has a small pith and prominent peripheral protoxylem points. The flattened megaspores had an original diameter of about 1 mm.

Disorganized microstrobili, agreeing with the megastrobili in size, and in the character of the axial stele and of the sporangium wall, contain numerous microspores 50 μ in diameter.

Whether these two kinds of strobili belong to the same species or not must await the discovery of more complete material. The lax nature of the cone and the absence of a downward projection of the lamina distinguish this from previously described species.

Sigillaria approximata Font. & White (8)

DIAGNOSIS.—Clathrarian type. Leaf bases tangentially elongated; vascular bundles and parichnos strands in upper half of scar. Primary xylem a complete ring inclosing a large pith. Corona markedly sinuate. Secondary xylem developed. Leaf traces without secondary xylem, at first mesarch, becoming tangentially elongated and with complete or nearly complete reduction of the centrifugal xylem, finally dividing into a double bundle in the leaf base. Middle cortex containing nests of sclerenchyma and sclerenchymatous sheaths about the leaf traces. Parichnos strands passing directly inward where they merge with the parenchyma accompanying the leaf traces. Small triangular peduncle scars occurring in verticils. Xylem strand of peduncle with peripheral protoxylem and without secondary thickening.

The stem is much flattened and slightly crushed, but portions of all tissues except the delicate ones immediately outside the xylem are clearly preserved (fig. 3). The specimen is about 25 cm. in length and its diameter exceeds 5.6 cm. The diameter of the stele is 1.8 cm.

Tangential sections through the leaf bases show that the stem is of the Clathrarian type (fig. 4). There are verticils of triangular organs (*p*, fig. 4) which are believed to be the scars where the peduncles of strobili were attached. These peduncle scars occur between longitudinal series of leaf bases whose shape they modify, but they do not appear to disturb the phyllotaxy. The shape and arrangement of the leaves and peduncles agree with ZEILLER's (15) figure of *Sigillaria approximata* and with FONTAINE and WHITE's (8) original description of impressions of this species.

The ring of primary xylem is continuous and is from 0.5 to 0.8 mm. in thickness. The outer margin is deeply and regularly undulate. Most of the large pith is destroyed by crushing (fig. 5). The secondary xylem forms a zone 0.3–0.6 mm. wide. The structure of both primary and secondary xylem agrees in all respects with that of *Sigillaria elegans* (10).

The leaf traces arise from the periphery of the primary xylem and invariably at the base of the furrows. When the leaf trace is about to separate from the primary xylem it consists of a circular group of from 25 to 50 tracheids (fig. 8). The bundle is mesarch, the smallest elements (protoxylem) being placed eccentrically toward the outer margin of the group. By the time the leaf trace emerges from the secondary xylem, it has elongated in the tangential direction (fig. 9). There is no secondary xylem in the leaf trace. The protoxylem is not actually seen at this level, but it is believed to have occupied part of the vacant space which is found in the center of the bundle. The bundle thus retains its mesarch structure but the centrifugal xylem is reduced. Farther up, the leaf trace becomes more and more tangentially elongated, with still more reduction of the centrifugal xylem, which may die out completely (fig. 10). The leaf trace passes through the secondary cortex in a horizontal direction, accompanied by an encircling zone of parenchyma (fig. 6). Within the leaf base it divides into a double bundle (*xx*, fig. 11).

No tissue is found in organic continuity with the exterior of the secondary xylem, the cambium and phloem not being preserved. The inner cortex consists of parenchyma, of which only fragments are preserved, and of numerous isodiametric sclerenchyma cells which occur both as scattered groups throughout the parenchyma and as sheaths surrounding the leaf traces.
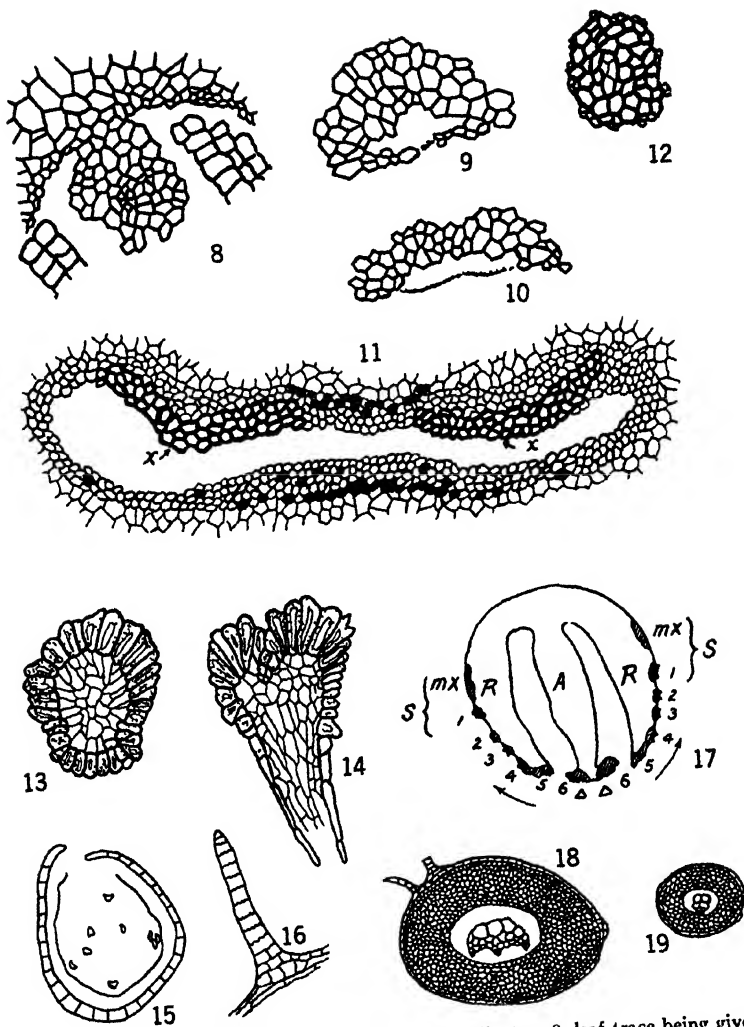
FIGS. 8-19 —Figs. 8-12, *Sigillaria approximata*, all ×75, 8, leaf trace being given off from border of primary xylem; 9, same in inner part of cortex; 10, same at outer margin of middle cortex; 11, same within leaf base (note double vascular bundle (*xx*) and gap below bundle caused by disintegration of delicate tissue); 12, bundle of peduncle. Figs 13-16, *Botryopteris americana*, all ×30 13, 14, transverse and longitudinal sections of sterile sporangium; 15, sporangium with spores; 16, equisetiform hair Fig. 17, diagram of branching of *B. forensis* (after BERTRAND) (*A*, central vascular band; *RR*, receptive pieces; ΔΔ, fundamental poles; 1 to 6, strands of protoxylem detached from poles and each destined for a secondary rachis; *SS*, masses destined for a secondary rachis). Each branch stele is formed by the union of a mass (*mx*) and a strand of protoxylem Figs. 18, 19, *Botryopteris* cf. *B. hirsuta* or *B. ramosa*, transverse sections of petioles ×12: 18, petiole with triarch bundle; 19, smaller petiole with monarch bundle.

The general form of the leaf bases has already been described. Occurring in the upper third of the leaf are the tangentially elongated vascular bundle and the two parichnos strands which are lateral to and slightly above the bundle (fig. 7). No ligule was recognized in any of the sections. Immediately below the vascular bundle is a space due to the disintegration of delicate tissues (fig. 11). The parichnos consists of delicate parenchyma, and its general appearance is that of aerenchyma. There is no differentiation into two zones as in *S. spinulosa*, but cells with dark contents are often found surrounding the parichnos. Internally the parichnos strands do not unite beneath the vascular bundle as in *S. spinulosa*, nor do they broaden out and finally surround the bundle as in *S. scutellata*, but continue unchanged into the inner part of the leaf base. In this region and in the outer part of the secondary cortex the parichnos changes its character and gradually merges with and becomes indistinguishable from the parenchyma which surrounds the leaf trace on its outward course through the secondary cortex (fig. 6).

The peduncle scars are roughly triangular in shape (*p*, fig. 4) and are much smaller than the leaf bases. They agree in general structure with those of *S. elegans*. The vascular bundle of the peduncle is roughly circular in section (fig. 12). The tracheids have very thick walls. Preservation is imperfect but the protoxylem is evidently peripheral. No leaf traces are given off by the peduncle bundle at this level. The cortex exhibits no special features. The tissues, in particular the conducting elements, are not so well preserved as are those of the leaf bases. This suggests that the strobili had fallen long before fossilization.

In external characters *S. approximata* is distinct from *S. elegans*, but the anatomy of their tissues is almost identical and this suggests a close relationship.

Sigillaria approximata is a Sub-Sigillarian species which exhibits characters formerly regarded as characteristic of the Eu-Sigillariae (1), namely, the possession of a continuous ring of primary xylem, monoxylic leaf traces (absence of secondary wood in the leaf trace), and a double leaf trace in the region of the leaf bases. This lends support to the doubts which have been expressed regarding the value of the sections Eu-Sigillariae and Sub-Sigillariae as indicative of actual differences (13).

Mazocarpon cf. *Mazocarpon shorense* Bens.

Several isolated megaspores, more or less reniform in section, were found. One specimen was still imbedded in sterile tissue (*sp*, fig. 20) still inclosed by a portion of the sporangial wall. Apart from the absence of any projections from the convex surface of the spore, these specimens are similar to and may be identified with *Mazocarpon shorense* (3).

Botryopteris **americana** sp. nov.

DIAGNOSIS.—Stele of petiole U-shaped in cross section, the three long xylem arms projecting abaxially. Pinna traces given off alternately from the terminal portion of the lateral xylem arms. Absence of fibrous sclerotic elements surrounding the xylem on its convex side and of gum canals in the cortex. The petiole bears equisetiform hairs. Sporangia and peculiar sterile sporangia as in *B. forensis*.

Sections of branching petioles were obtained. The larger of the petioles (fig. 21) has a diameter of 5 mm. Its structure is very similar to that of *B. forensis* Renault (11, 12). The following differences were noted: the absence of a sheath of thick walled cells, almost fibrous in nature, surrounding the xylem on its convex side; and the absence of gum canals in the cortex.

Certain isolated structures and organs, occurring abundantly in the same coal ball as the petioles, may be ascribed with certainty to *Botryopteris americana* on account of their agreement with similar structures borne by *B. forensis*. These are specialized sterile sporangia (figs. 13, 14), sporangia with spores (fig. 15), and equisetiform hairs (fig. 16).

At the end of the central xylem arm of the petiole are borne two crescentic groups of protoxylem, the "fundamental poles" of BERTRAND (6). According to BERTRAND and CORNAILLE (4), these give rise to groups of protoxylem which pass laterally to the tip of the lateral xylem arms. These protoxylem groups shift their position along the external face toward the base of the arm. Owing to poor preservation it was not possible to see this in the writer's specimens. BERTRAND's (6) diagram of the course of the protoxylem bundles in branch formation is reproduced (fig. 17). In the writer's sections

there are two cases of branching and in both the method of branch formation differs from that shown by BERTRAND. The terminal portion of the xylem arm, bearing all the protoxylem groups, separates to form the lateral branch (fig. 21) instead of only a small portion of xylem from the side of the arm as shown by BERTRAND. The mode of origin of the pinna trace is thus similar to that of *B. antiqua* (2).

Botryopteris cf. *B. ramosa* Will. & *B. hirsuta* Will.

There are several small petioles in a poor state of preservation. The largest has a diameter of about 1.5 mm. The vascular strand is crescentic, with three small projections on the concave side (fig. 18). Smaller petioles have a monarch bundle (fig. 19). Specific identification is not possible, but the petioles agree in structure with those of *B. ramosa* or *B. hirsuta*.

Anachoropteris **clavata** sp. nov.

DIAGNOSIS. Stele of petiole horseshoe-shaped in cross section, the arms pointing abaxially and expanding distally. Protoxylem groups occurring as projecting points along the adaxial side of the stele. Cortex not projecting between the xylem arms. Pinna traces given off alternately from the external face of the xylem arms.

Transverse and longitudinal sections of a branching petiole were prepared. In transverse section the unbranched petiole measures 2.8 by 1.7 mm. The stele is a large U-shaped bundle with the arms of the U much swollen and club-shaped (fig. 22), from which character the species derives its name. From the outline of the petiole it appears that the xylem arms pointed away from the stem.

The largest tracheids are in the arms of the U and the smaller tracheids are found toward the base. There are about six projecting protoxylem points along the convex side of the base, the petiole thus being endarch. The protoxylem occurs as distinct projecting points and not as projecting crescentic cusps as in *A. decaisnii* (5). The tracheids of the protoxylem points have close spiral or scalariform pitting. The metaxylem elements have crowded elliptical bordered pits arranged in spiral sequence on all walls.

The cortex consists of moderately thick walled cells elongated parallel to the axis. The cells become progressively longer and nar-

rower toward the exterior. There is no tongue of cortex projecting between the xylem arms as in previously described species of the genus All the cortical cells have transverse end walls Sclerenchyma and gum canals are absent The epidermis bears short stout multi-cellular hairs
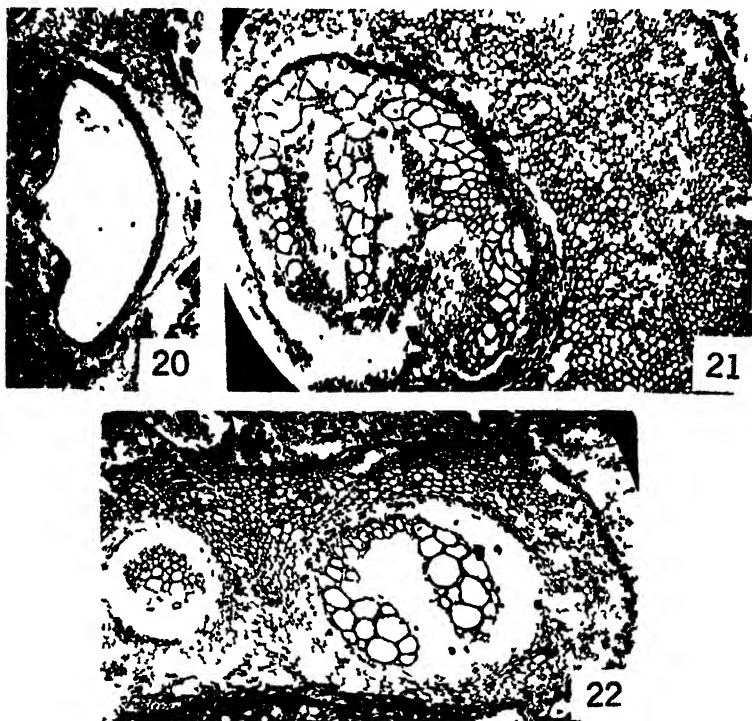
FIGS 20–22 Fig 20, *Mazocarpon* sp , megaspore and portion of sporangium (*sp*), ×20 Fig 21, *Botryopteris americana*, transverse section of petiole Note distal portion of lateral arm beginning to separate off to form a branch stele, ×20 Fig 22, *Ana choropteris clavata*, transverse section of branching petiole, ×20

The method of branch formation is the same as in *A decaisnii*

Anachoropteris clavata is distinct from any of the previously described species of that genus, in possessing protoxylem points instead of crescentic cusps, in the much expanded xylem arms, and in the absence of a tongue of cortex projecting between the xylem arms.

Cardiocarpus **spinatus** sp. nov.

DIAGNOSIS.—Platyspermic seeds with double vascular system. Outer vascular system separates from the chalazal bundle before the latter pierces the sclerotesta. Sclerotesta thick, with spiny outgrowths. Size about 6.5 mm. long by 8 mm. wide by 3.5 mm. thick.

Longitudinal sections were made of two seeds, and transverse sections of the half of one (figs. 23, 24). The seeds are decidedly platyspermic. One measures 6.5 mm. long by 8 mm. wide by 3.5 mm.
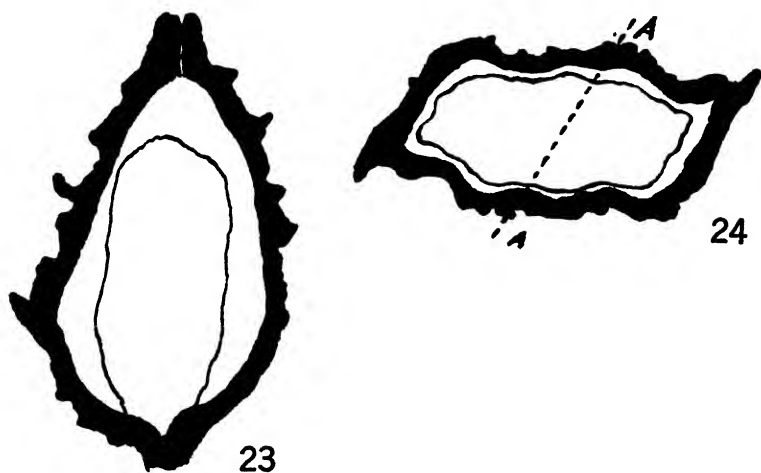
Figs. 23, 24. *Cardiocarpus spinatus:* Fig. 23, longitudinal section; ×8. Fig. 24, transverse section (.1-.1, position of section illustrated in fig. 23); ×8.

thick; the other is slightly larger. The thickest part of the seed is about one-third the distance from the base to the summit. The base of the seed is rounded; the apex is produced into a short and narrow micropylar canal. In transverse section the seed is lenticular, and bears two ribs in its principal plane. These descriptions and measurements refer to the exterior of the sclerotesta, since the outer fleshy layer, the sarcotesta, has been almost completely destroyed.

The moderately thick sclerotesta consists of two layers of tissue, an inner layer of moderately thick walled cells elongated in the direction of the axis of the seed, and an outer layer of much thicker walled, more or less isodiametric cells. This outer layer is of vari-

able thickness and has spines at its outer surface. These projections may not have been visible, being originally imbedded in the fleshy portion of the seed.

The nucellus is very poorly preserved, only the superficial layer of large polygonal platelike cells remaining. No pollen chamber is discernible, possibly because of the poor state of preservation, nor was it possible to determine how much of the nucellus was free from the integument. The megaspore membrane is preserved.

The vascular system of the seed is double, consisting of an outer system within the sarcotesta and an inner system within the nucellus. The outer system consists of a bundle of delicate tracheids running longitudinally the full length of the seed along the outer margin of each of the two sclerotestal ribs. As far as could be determined from the unfavorable planes of section, this outer system separates from the chalazal bundle before the latter pierces the sclerotesta. The inner system consists of the continuation of the main bundle which pierces the sclerotesta at the chalaza and expands into a plate of transfusion tissue in close contact with the megaspore membrane within the base of the nucellus. There is no indication of the inner vascular system extending up the sides of the nucellus.

The platyspermic form of this seed, and its double vascular system (the outer set of bundles are given off from the chalazal bundle before the latter pierces the sclerotesta) are characters which place it in the genus *Cardiocarpus*. It is a well marked species and is less than half the size of any previously described. In the possession of a thick stony testa it resembles *C. sclerotesta* Br. (7), but differs from that species in the less sclerotic nature of the inner layers of the integument and in the spiny outgrowths of the exterior of the sclerotesta.

Calamites sp.

Roots and rootlets.

Sphenophyllum plurifoliatum Will. & Scott

Stem.
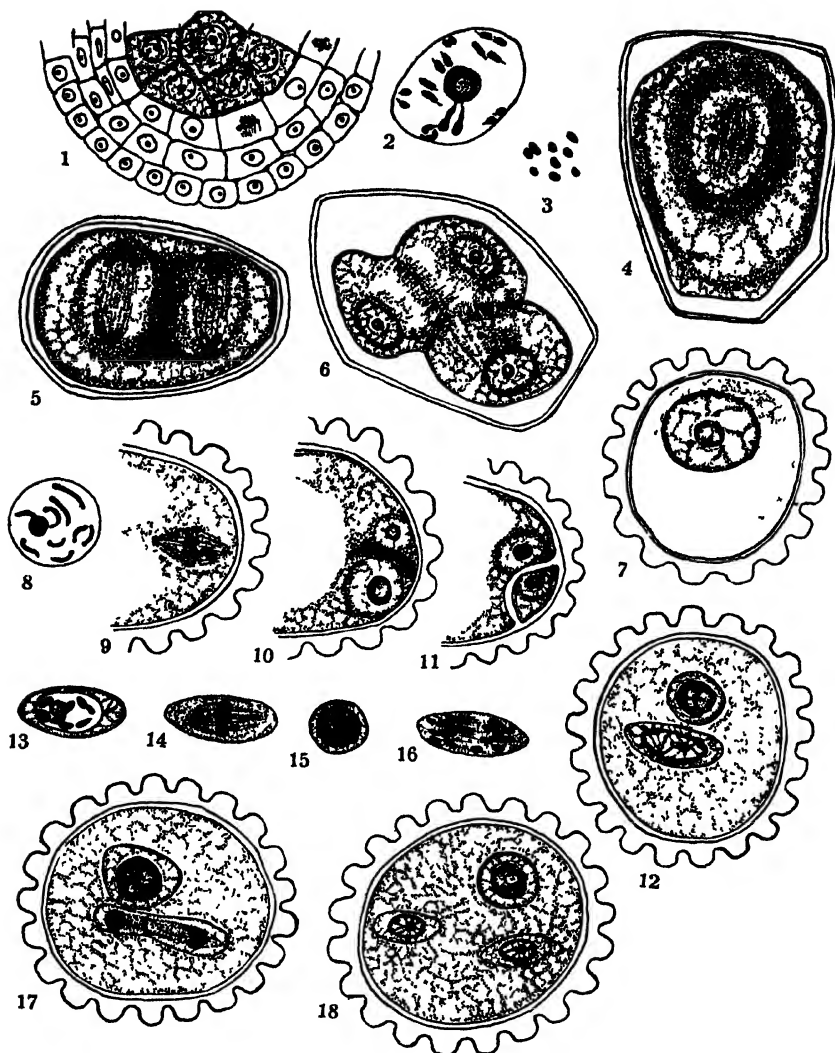
FIGS 1–18 *Kochia trichophylla* fig 1, longitudinal section through portion of anther showing microspore mother cells and mitotic figures in tapetal cells, fig 2, late diakinesis showing nine pairs of chromosomes, one pair apparently attached to nucleolus; fig 3, polar view of heterotypic equatorial plate showing nine chromosomes, fig 4, heterotypic anaphase, cytoplasmic zone surrounding the spindle, and granular zone at periphery of cell; fig. 5, homoeotypic telophase showing cytoplasmic and granular zones, fig. 6, microspore formation, daughter nuclei distinct, and cell plate formation shown by thickenings on spindle fibers; fig. 7, microspore showing wall sculpturing; large vacuole present in cytoplasm; fig. 8, microspore nucleus with nine chromosomes; fig 9, equatorial plate stage of microspore nucleus; figs. 10, 11, cell plate formation and maturation of tube and generative cells; fig. 12, young pollen grain with tube and generative cells; figs. 13-17, stages in division of generative cell, fig 18, pollen grain with two male gamete cells within the tube cell Fig. 1, ×1000; figs 2-18, ×2000.

comes to lie at one side of the cell, imbedded in dense cytoplasm. A large vacuole fills most of the remainder of the space within the microspore wall. The nucleus divides, its chromatin becomes aggregated into a spireme, and later nine chromosomes can be seen (fig. 8). A spindle is now formed at right angles to the wall of the micro· spore, one pole being closely adjacent to this wall (fig. 9). Thickenings appear on the spindle fibers midway between the nuclei, formed as a result of the division of the microspore nucleus, and a cell plate is formed (fig. 10). As a result of this division the microspore is divided into a small generative cell and a much larger tube cell. The two cells are surrounded by thin membranes (fig. 11).

The nucleus of the tube cell is spherical; its nucleolus is large and its cytoplasm granular with very small vacuoles. The ovoid generative cell with its ovoid nucleus enlarges and becomes imbedded in the cytoplasm of the tube cell (fig. 12). The generative nucleus divides and a cell plate is formed equidistant between the two daughter nuclei separating the generative cell into male gamete cells (figs. 13–17). In the mature pollen grain the tube cell cytoplasm stains heavily whereas the cytoplasm immediately surrounding each of the male gamete nuclei stains lightly and a definite membrane seems to separate the two types of cytoplasm. The tube nucleus does not continue to enlarge whereas there is some further growth of the pollen grain (fig. 18).

Kochia scoparia

Nine pairs of chromosomes are present in diakinesis stages of K. scoparia (fig. 19). One of the larger pairs is apparently attached to the nucleolus in a manner similar to that already described for K. trichophylla. Granular cytoplasmic zones surround the spindles of both the heterotypic and homoeotypic divisions, although they are not so distinct as those observed in K. trichophylla (figs. 20–22). The nine chromosomes seen in a polar view of the heterotypic equatorial plate are similar in size and shape (fig. 21), there being little evidence of the variations earlier found at diakinesis. The microspore mother cells in K. scoparia are somewhat smaller than those of K. trichophylla. A marked difference in the sizes of the mature pollen grains of these species was also observed, those of K. scoparia being the

smaller of the two. Otherwise, as seen in sectional view, they are
similar. The wall of the pollen grain is so sculptured as to have
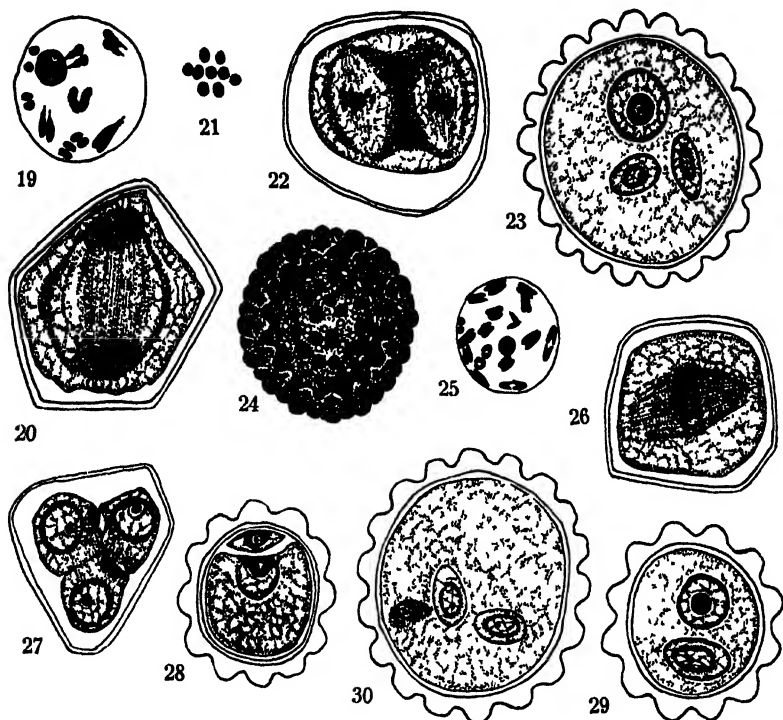
FIGS 19 30   Figs 19 24 *Kochia scoparia.* fig 19, late diakinesis, nine pairs of
chromosomes present, fig 20, heterotypic telophase showing cytoplasmic zone and slight
granular zone at periphery of cell, fig 21, polar view of heterotypic telophase (nine
chromosomes present), fig 22, homoeotypic equatorial plates with surrounding cyto-
plasmic zones, fig 23, pollen grain with two male gamete cells and tube cell, fig 24,
pollen grain, surface view Figs 25 30, *Chenopodium hybridum* fig 25, late diakinesis
showing 18 pairs of chromosomes, fig 26, heterotypic equatorial plate (note absence of
dense cytoplasmic zone), fig 27, microspore formation (cell plate indicated by thicken-
ings on spindle fibers midway between daughter nuclei), fig 28, two-celled pollen grain,
fig 29, pollen grain with tube and generative cells, fig 30, pollen grain with two male
gamete cells and disintegrating tube nucleus × 2000

marked depressions both as seen in sectional (fig. 23) and in surface
view (fig. 24). At the bottom of each of these depressions the wall is
very thin.

Chenopodium hybridum

The chromosomes as seen at diakinesis are ovoid in shape and show little variation in size (fig. 25). There are 18 pairs. A distinct spindle is present during the heterotypic division and there is no evidence of cytoplasmic or granular zones such as are found in *Kochia* (fig. 26). At microspore formation the nuclei are connected by the spindle fibers of the homoeotypic and heterotypic divisions. Thickenings appear on the fibers midway between the nuclei. The cell plate splits and ingrowing septa, which are newly forming cell walls, cut across the spindle and separate the four microspores (fig. 27). Whether this is followed or accompanied by furrowing could not be determined, but it is suggested that, since osmotic changes are occurring at all times within the cell, the effect of furrowing is produced by the spore assuming a spherical shape. The splitting of the cell as seen in figure 27 would indicate that the ingrowing septa are the result rather than the cause of microspore formation. The development of the pollen grain follows much the same order as in *Kochia* (figs. 28–30).

The mature microgametophyte contains two male gamete cells and a tube nucleus that is in an advanced stage of disintegration.

Chenopodium album

Eighteen pairs of irregularly shaped chromosomes are present at diakinesis in *C. album*, and as in the species of *Kochia* already described, one of the larger pairs appears to be attached to the nucleolus (fig. 31). Cytoplasmic and granular zones such as found in the meiotic divisions of *Kochia* are absent in this species (fig. 32). The chromosomes as seen in a polar view of the heterotypic telophase are 18 in number and regular in shape (fig. 33). The microspores are separated by cell plates and the development of the microgametophyte is essentially the same as that described for *C. hybridum* (figs. 34–37).

Atriplex patula var. hastata

Diakinesis shows 18 pairs of chromosomes all of which are similar in size and shape (fig. 38). The granular cytoplasmic zone is absent but there is a small zone of denser cytoplasm about each spindle (fig. 39). Eighteen chromosomes can be counted on the spindle seen

in the polar view in this figure The mature pollen grain has two
male gamete cells, the tube nucleus is in a stage of disintegration.
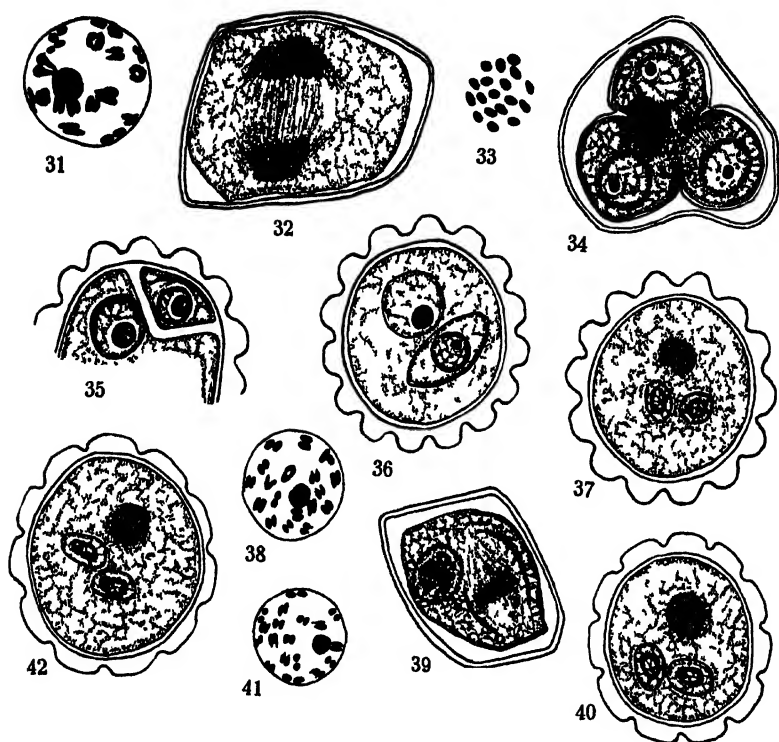
FIGS 31 42 – Figs 31 37 *Chenopodium album* fig 31, late diakinesis showing 18
pairs of chromosomes, fig 32 heterotypic telophase, fig 33 heterotypic telophase,
polar view showing 18 chromosomes, fig 34 microspore formation showing splitting
of cell plate and ingrowing septa, fig 35 portion of young two celled pollen grain,
fig 36, pollen grain with tube and generative cells, fig 37 pollen grain with two male
gamete cells and disintegrating tube nucleus Figs 38 40 *Atriplex patula* var *hastata*
fig 38, late diakinesis showing 18 pairs of chromosomes, fig 39, homoeotypic spindles
at right angles to each other with cytoplasmic zone present and 18 chromosomes
countable on one equatorial plate, fig 40 pollen grain with two male gamete cells and
disintegrating tube nucleus Figs 41 42 *Salsola kali* fig 41, late diakinesis showing 18
pairs of chromosomes, fig 42, pollen grain with two male gamete cells and disintegrating
tube nucleus ×2000

The rounded portions of the pollen grain wall are considerably
broader and more flattened than in the species already described
(fig 40).

Salsola kali

The haploid chromosome number in *Salsola kali* is 18. A long pair of chromosomes lie closely adjacent to the nucleolus at diakinesis (fig. 41). The sculpturing on the wall of the pollen grain is similar to that of *Atriplex patula* var. *hastata,* in that there are fewer depressions and the raised portions of the wall are broad and somewhat flattened (fig. 42).

Discussion

The haploid chromosome numbers herein reported but not previously recorded, in so far as the writer is aware, are as follows: *Kochia trichophylla* 9, *K. scoparia* 9, *Salsola kali* 18, and *Atriplex patula* var. *hastata* 18. WINGE (11) likewise found 18 pairs of chromosomes in *A. patula* but only nine pairs in *A. hastata.* ROSENBERG (7) recorded about 24 as the diploid number of chromosomes in *A. hastata.* In *A. hymenelytra,* BILLINGS (2) found nine to be the haploid number in some daughter cells and ten in others.

Chenopodium hybridum and *C. album* each have 18 pairs of chromosomes. WINGE (11) reported nine as the haploid number in each of these species.

The presence of cytoplasm and granular zones was observed in the heterotypic and homoeotypic divisions in both *Kochia* species and in *Atriplex.* They were not found in *Chenopodium hybridum, C. album,* or *Salsola kali;* nor were they recorded by TUSCHNJAKOWA (10) in *Spinacea oleracea* or by ARTSCHWAGER (1) in *Beta vulgaris.* The presence of such zones, however, is not uncommon in other angiosperms.

The formation of the microspores by cell plate formation and not by furrowing, as has been described by early workers, is characteristic of *Kochia trichophylla, Chenopodium hybridum,* and *C. album.*

The behavior observed in the divisions of the generative cell and its nucleus to form the two male gametes suggests very definitely the formation of a cell plate.

Male cells are present in each of the species studied but are larger in *Kochia.* The tube nuclei disintegrate early and in mature pollen grains they appear to be diffusing into the surrounding cytoplasm

(figs. 30, 37, 40, 42). TUSCHNJAKOWA (10) in *Spinacea* shows the tube nucleus beginning to disintegrate whereas ARTSCHWAGER (1) makes no mention of this in *Beta*.

Summary

1. The primary sporogenous cells in the members of the Chenopodiaceae examined divide to form several rows of microspore mother cells.

2. Prior to the nuclear divisions of the microspore mother cells the tapetal cells divide mitotically and each cell becomes binucleate.

3. The heterotypic spindle is surrounded by cytoplasmic and granular zones in *Kochia* and *Atriplex*. Such zones are not present in *Chenopodium*. This stage was not observed in *Salsola*.

4. Remains of the heterotypic and the homoeotypic spindles persist so that the tetrad nuclei are connected by two prominent spindles and four less distinct spindles.

5. Paired thickenings appear on the cytoplasmic strands midway between each of the nuclei. The cell plate splits and a wall is laid down so that ultimately four microspores are formed.

6. The microspore nucleus migrates to the periphery of the cell and there divides to form the generative and tube nuclei. By means of cell plate formation midway between these two nuclei, two cells of equal size are formed.

7. The generative cell ultimately becomes imbedded in the cytoplasm of the tube cell.

8. The generative cell divides to form two male gametes. Each gamete consists of a nucleus and a definite layer of cytoplasm delimited by a distinct membrane.

9. The tube nucleus of the mature pollen grain shows evidence of disintegration in all the species examined with the exception of the two species of *Kochia*.

10. The haploid number of chromosomes is as follows: *Kochia trichophylla* 9, *K. scoparia* 9, *Chenopodium hybridum* 18, *C. album* 18, *Atriplex patula* var. *hastata* 18, *Salsola kali* 18.

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LITERATURE CITED

1. ARTSCHWAGER, E., Development of the flowers of the sugar beet. Jour. Agr. Res. **34**:1-25. 1927.

2. BILLINGS, F. H., Male gametophyte of *Atriplex hymenelytra*. BOT. GAZ. **95**:477–484. 1934.

3. COOPER, D. C., Microsporogenesis and the development of the male gametes in *Portulaca oleracea*. Amer. Jour. Bot. **22**:453–459. 1935.

4. FINN, W. W., Male cells in angiosperms. I. Spermatogenesis and fertilization in *Asclepias Cornuti*. BOT. GAZ. **80**:1-25. 1925.

5. FRYE, T. C., A morphological study of certain Asclepiadaceae. BOT. GAZ. **34**:389–413. 1902.

6. PODDUBNAJA-ARNOLDI, V., Spermazellen in der Familie der Dipsacaceae. Planta **21**:381–386. 1933.

7. ROSENBERG, O., Über den Bau des Ruhekerns. Svensk Bot. Tidskr. **3**:163–173. 1909.

8. SHATTUCK, C. H., A morphological study of *Ulmus americana*. BOT. GAZ. **40**:209-223. 1905.

9. STOMPS, THEO J., Kernteilung und Synapsis bei *Spinacea oleracea* L. Biol. Centralbl. **31**:257–309. 1911.

10. TUSCHNJAKOWA, M., Untersuchungen uber die Kernbeschaffenheit einiger diozischer Pflanzen. Planta **7**:427–443. 1929.

11. WINGE, O., The chromosomes, their number and general importance. Compt. Rend. Trav. Lab. Carlsberg **3**:131–275. 1917.

12. WYLIE, R. B., The morphology of *Elodea canadensis*. BOT. GAZ. **75**:191–202. 1904.

13. ———, Sperms of *Vallisneria spiralis*. BOT. GAZ. **75**:191-202. 1923.

ORIGIN OF THE FRINGE TISSUE OF THE COTTON SEED[1]

R. G. REEVES

(WITH EIGHT FIGURES)

Introduction

The delicate white sheath inclosing the embryo of the cotton seed has attracted attention since the cotton seed first came into prominence as a commercial product. It has been studied only incidentally, however, by those who have investigated the structure of the cotton seed.

This sheath was found by HANAUSEK (4) to consist of two tissues, the outer of which he designated as fringe cells (Franzenzellen). HANAUSEK agreed with HARZ (5) that this is the remains of the perisperm. He showed the inner portion of the sheath to be endosperm. BRETFELD (3) described the outer portion of this sheath as "dem Knospenkern hervorgegangenen Schichte." VAN ZWALUWEN-BURG and SCHLOTTERBECK (9), in their discussion of the entire membrane which separates the inner pigment layer from the embryo, state that it is "composed of the obliterated nucellus and endosperm, and the inner epidermis of the inner integument." WINTON (7) referred to the tissue as perisperm, thereby indicating that it is the remaining tissue of the nucellus. BALLS (1) concluded that all of the cells of the inner integument lying within the palisade layer are disorganized and that their fragmentary remains form the second pigment layer. He stated further that the inner epidermis of the inner coat (inner integument) disappears with the parenchymatous cells in marked contrast to the enormous palisade layer formed from the outer epidermis.

In a previous report which included a brief discussion of the fringe tissue, REEVES and VALLE (6) explained it as the remains of the nucellus, as HARZ, HANAUSEK, BRETFELD, WINTON, and BALLS had previously done. The tissue was accordingly designated as peri-

sperm. Since that time, however, this tissue has been the subject
of a more detailed study, and the evidence concerning its origin is
now conclusive.

Methods

While studies of comparative anatomy of seeds of malvaceous
plants were in progress, several observations were made which indi-
cated that the fringe tissue originates as the inner epidermis[2] of the
inner integument. This layer of cells was therefore definitely identi-
fied in very young ovules, and followed through successively older
stages to the mature condition.

The material was imbedded in paraffin and stained with Dela-
field's haematoxylin.

Observations and results

When mature seeds are stored the embryo often shrinks until it
becomes loose in the hull. Under such conditions the fringe tissue
adheres to the endosperm and the two usually separate from the
hull, remaining attached to the embryo. At maturity the fringe
tissue is highly differentiated. Its cells are devoid of contents, and
their walls are composed of lignin and suberin. The layer is thicker
near the micropyle than elsewhere.

In ovules fixed soon after fertilization the outer layer of the inner
integument was easily traced completely through the endostome
on both sides of the foramen, as it appeared in sections. Here the
layer of cells under observation was seen to be continuous (fig. 1).
It was thus possible to identify definitely the inner epidermis of the
inner integument. The epidermis was difficult to trace through the
endostome except in the earliest stages, for as the ovule develops
the epidermis becomes less prominent through the endostome. The
identity of the epidermal layer was further determined by locating
the cuticle. VAN WISSELINGH (8) showed that, although the cuticle
between the integuments is lost rather early, that between the inner
integument and the nucellus remains and even becomes thicker.
In the present study, the cuticle was easily recognized just beneath
the layer here regarded as the inner epidermis.

[2] The terms inner epidermis and outer epidermis are used here for convenience, it
being fully understood that the integument is completely surrounded by a single
epidermis.

In this early stage of development, the cells of the inner epidermis are small, dark-staining, and have large nuclei. The next significant stage shows the inner epidermis of the inner integument well on its
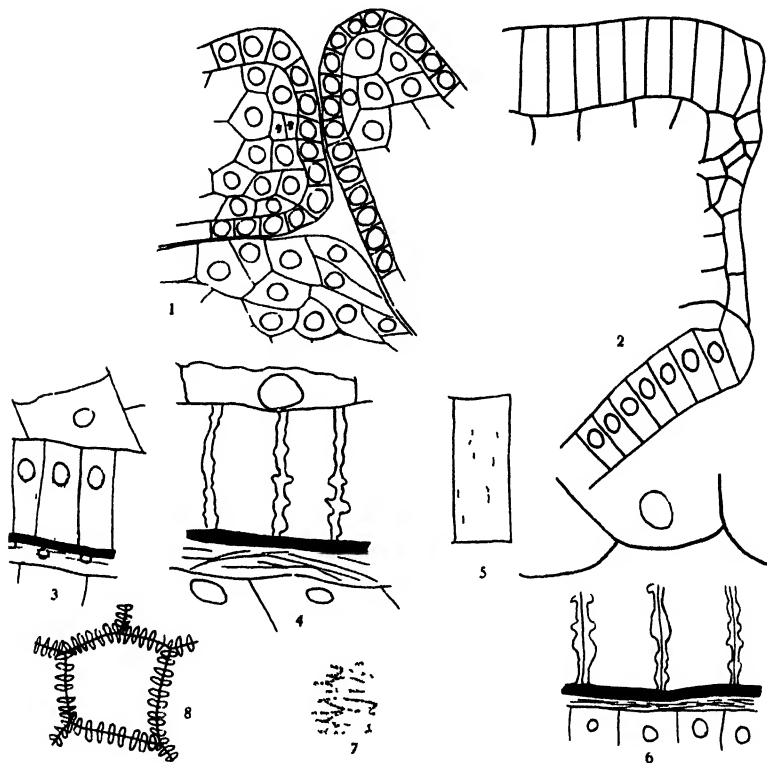
FIGS 1–8   Fig 1, section through endostome of young cotton ovule showing epidermises of the two integuments, ×450 Fig 2, section of slightly older inner integument at endostome showing beginning of differentiation of both epidermises; ×450. Fig. 3, fringe cells showing first signs of markings on walls, ×865. Fig 4, longitudinal section of slightly older fringe cells showing absence of protoplasm; ×865 Fig. 5, same, surface view; ×865 Fig 6, longitudinal section of nearly mature fringe cells showing their separation from the tissue external to themselves; ×865. Fig. 7, lateral view of markings on wall of mature fringe cell; ×1170 Fig. 8, section through mature fringe cell showing details of markings on walls, ×865.

way in development (fig. 2). The cells of both the inner and the outer epidermis elongate considerably and for a time almost equally. The cells of the inner epidermis elongate first near the micropyle;

those successively farther toward the chalaza elongate later. The cells in the region of the chalaza, however, never reach the extreme lengths of those nearer the micropyle. This accounts for the greater thickness of the fringe tissue near the micropyle. This is the layer of cells regarded by BARRITT (2) as inner palisade cells.

When the cells are from twice to three times as long as wide they cease to elongate. At this time their nuclei are inclined to lie in the end directed outward, and their protoplasm is still very dense.

Markings occur on the cell walls of the inner epidermis early in the development of the tissue, being first observed at about the time when the nuclei begin to migrate outward (fig. 3). They become more prominent as the seed develops, and at maturity are very conspicuous.

Shortly after the nuclei migrate outward, the cytoplasm shows signs of disintegration. When it is partially disintegrated, the nuclei break down and disappear. Long before the seed is mature, the cells of the inner epidermis become entirely devoid of contents (figs. 4, 5).

During this time there is evident resorption of cells in the parenchyma tissue of the inner integument (fig. 6). This resorption begins along the external side of the epidermis, and soon disconnects the epidermis from the remaining parenchyma. This fact explains why the fringe tissue is never found attached to the inner integument.

The peculiar markings on the walls of mature fringe cells have been briefly discussed by previous investigators. BRETFELD (3) described them as being nodular and wavy. HANAUSEK (4) stated that the tissue consists of polyhedric cells whose walls are especially fringed, highly ramified, and proliferated. VAN ZWALUWENBURG and SCHLOTTERBECK (9) noted that the cells of this membrane have walls thickened irregularly, rendering the outlines of the cells wavy and indistinct. VAN WISSELINGH (8) observed that in cross sections this tissue has radial marks upon the cell walls, and in tangential sections the marks are seen to be caused by characteristic thickenings upon the rest of the thin cell walls. Apparently the descriptions given by these investigators embody our best knowledge up to the present time of the structure of this tissue.

A slight similarity between these markings and those of the palisade cells (outer epidermis) was observed, but this similarity is only superficial. The markings of the inner epidermis are seen to be reticulate in arrangement when observed under high magnification (fig. 7), while those of the outer epidermis are parallel.

The thin areas left among the thickenings of these cell walls appear to be very similar to, if not identical with, simple pits. On close examination of well prepared sections at high magnification, they appear to be areas in which no secondary wall was laid down. Further, the thin places on the walls of adjoining cells usually coincide; that is, they are opposite each other (fig. 8). Slight variation was apparent in the details of this modification of cell wall structure. This apparent variation may be accounted for, however, by the fact that the true nature of the markings was seen only under the most favorable conditions.

Outer tangential walls were never observed, and inner tangential walls appeared to be lacking also. Whenever the tissue was separated from that adjoining on both sides, no tangential walls, either inner or outer, were ever observed. Since under natural conditions the fringe layer always separates from the inner pigment layer (which lies above it) and adheres to the layers below, the cuticle beneath this layer occupies the place of an inner tangential wall.

Of the nucellus, only the cuticle and a few fragments remain at the time of maturity. The cuticle is closely associated with and indistinguishable from the cuticle of the inner epidermis of the inner integument.

Summary

The tissue of the cotton seed often termed perisperm is here shown to originate as the inner epidermis of the inner integument. This term, however, is properly applied to tissues which originate from the nucellus only, and its misuse in the past when applied to the cotton seed is the result of a lack of information concerning its origin. HANAUSEK's name Franzenzellen does seem appropriate, however, and has already been translated into English as "fringe cells" (7). The names fringe cells and fringe tissue seem entirely satisfactory.

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LITERATURE CITED

1. BALLS, W. L., The development and properties of raw cotton. Black, London. 1915.
2. BARRITT, N. W., The structure of the seed coat of *Gossypium* and its relation to the growth and nutrition of the lint hairs. Ann. Bot. **43**:483–489. 1929.
3. BRETFELD, F., Anatomie des Baumwolle- und Kapoksamens. Jour. Landw. **35**:29–56. 1886.
4. HANAUSEK, T. F., Zur mikroskopischen Charakteristik der Baumwolle-producte. Zeitschr. Allgem. Österr. Apoth. Ver. **26**:569–572; 591–595. 1888.
5. HARZ, C. D., Landwirtschaftliche Samenkunde. II. Parey, Berlin. 1885.
6. REEVES, R. G., and VALLE, C. C., Anatomy and microchemistry of the cotton seed. BOT. GAZ. **93**:259–277. 1932.
7. WINTON, A. L., The anatomy of certain oily seeds with special reference to the microscopic examination of cattle foods. Connecticut Agr. Exp. Sta. Rep. 175–198. 1903.
8. WISSELINGH, C. VAN, Bijdragen tot de kennis van de zaadhuid. Twaalfde bijdrage: Over de zaahuid der Malvaceeën en Bombacaeeën. Pharmac. Weekbl. Nederland **58**:1149–1167. 1921.
9. ZWALUWENBURG, A. VAN, and SCHLOTTERBECK, J. O., Developmental history of important seeds. Proc. Amer. Pharm. Assoc. 185. 1889.

CERTAIN NOVELTIES IN BIDENS L. AND COREOCARPUS BENTH. (COMPOSITAE)

EARL EDWARD SHERFF

COREOCARPUS ARIZONICUS **macrophyllus** var. nov.—Folia majora, saepe 9–11.5 cm. longa, segmentis plus minusve atro-punctatis et plerumque 1.5–2 mm. latis, terminali saepe 6–8 cm. longo.

Specimens examined: *Dr. Edward Palmer* 294, southwestern Chihuahua, Mexico, August–November, 1885 (type, Herb. U.S. Nat.: cotypes, Herb. Gray; Herb. N.Y. Bot. Gard.).

Bidens coreocarpoides sp. nov.—Herba glabrata, ramosa, erecta e radice simplici tamen forsan perennis, ∓3 dm. alta, ramis tenuibus suberectis. Folia non perspicua, opposita, pinnatim 3- vel 5-partita vel summa simplicia, breviter petiolata petiolis plus minusve his-pido-ciliatis 2–8 mm. longis, petiolo adjecto 1.5–3 cm. longa, seg-mentis rhachi similibus linearibus crassiusculis acriter indurato-apiculatis 0.4–1 mm. latis. Capitula elongato-pedunculata peduncu-lis subcorymbose dispositis tenuissimis saepius nudis plerumque 8–12 cm. longis, radiata, pansa ad anthesin circ. 1.5- 1.8 cm. lata et circ. 6–7 mm. alta. Involucrum glabrum, bracteis exterioribus circ. 8, appressis, oblongo-linearibus, apice subacuto induratis, quam interioribus oblongo-ovatis dimidio brevioribus. Flores ligulati ple-rumque 8, flavi, circ. 7-nervati, ligula plus minusve oblongi, apice denticulati, 7-9 mm. longi. Flores disci sub 3 mm. longi. Ovaria plana, oblongo-linearia vel cuneato-linearia, corpore ∓1.5 mm. longa, apice biaristata aristis retrorsum hamosis.

Specimens examined: *E. W. Nelson* and *E. A. Goldman* 7389, alt. 50–200 feet, from Cape San Lucas to San Jose del Cabo, southern-most Baja (Lower) California, Jan. 4, 1906 (type, Herb. U.S. Nat.).

In habit somewhat suggestive of *Coreocarpus arizonicus* (A. Gray) Blake.

Coreocarpus shrevei sp. nov.—Herba annua, simplex vel sub-erecte pluriramosa, glabra vel glabrata, 3–5 dm. alta, caule ramisque tenuissimis. Folia subsessilia vel petiolata petiolis tenuibus 0.5–2.5 cm. longis, petiolo adjecto 1.5–4 (rarius –5.5) cm. longa, 2–3 pinna-

tifida; segmentis ultimis linearibus vel oblongis, membranaceissimis, non punctatis, apice acuto vel obtuso mucronulatis. Capitula tenuissime pedunculata (pedunculis saepius 1–3 ad utrumque ramum, saepe 1–3-subulato-bracteolatis, saepius 2–8 cm. longis, interdum filiformibus), ad anthesin 1.3–2.5 cm. lata et 4.5–6 mm. alta; cum fructibus 6–9 mm. latis et 3.5–4.5 mm. altis. Involucrum campanulatum vel late cylindricum; bracteis oblongis vel ovatis vel obovatis, apice plus minusve acuminatis, 3.5–4.7 mm. longis. Flores ligulati 4–7 (saepius 5), rosacei (praecipue secundum ∓4 venas) vel flavidi; tubo glabro quam involucro breviore; ligula oblonga vel obovata, saepe acri-denticulata vel -emarginata, 5–10 mm. longa. Paleae lineares vel oblongae, plerumque acutae, floribus tubulosis breviores sed achaeniis longiores. Disci flores aurantiaci, 2.2–2.8 mm. longi. Achaenia iis *Coreocarpi parthenioidis* similia sed minora (exteriora corpore 2–2.5 mm. longa) et alis saepe altius incisa (dentibus saepe manifeste separatis et saepe cuneatis).

Specimens examined: *A. W. Anthony,* Santo Domingo, Baja California, Feb. 23 (Herb. Univ. Calif.); *T S. Brandegee,* Magdalena Bay, Baja California, January, 1889 (Herb. N.Y Bot. Gard.); *idem,* Purisima, Baja California, Feb. 18, 1889 (Herb U.S. Nat.); *G. N. Collins, T. H. Kearney,* and *J. H. Kempton* 148, Isla Partida, Baja California, Apr. 1, 1931 (Herb. Field Mus.); *iidem* 152, *eodem loco et tempore* (Herb. U.S. Nat.); *Marcus E. Jones* 23365, Guaymas, State of Sonora, Mexico, Jan. 26, 1927 (Herb. Univ. Calif.); *idem* 24174, Laguna Mts., Baja California, Mar. 2, 1928 (Herb. Univ. Calif.); *Dr. Edward Palmer* 19, La Paz, Baja California, Jan. 20–Feb. 5, 1890 (Herb. Gray; Herb. N.Y. Bot. Gard.; Herb. U.S. Nat.); *idem* 248, Santa Agueda, Baja California, Mar 4–6, 1890 (Herb. Gray; Herb. U.S. Nat.); *idem* 660, mountain sides, Los Angeles Bay, Baja California, Oct. 12, 1887 (Herb. Gray; Herb. N.Y. Bot. Gard.; Herb. U.S: Nat.); *idem* 795, Lagoon Head, Baja California, Mar. 6–15, 1889 (Herb. Univ. Calif., 2 sheets; Herb. Gray; Herb. N.Y Bot. Gard.; Herb. U.S. Nat.); *C. A. Purpus* 74, alt. 1300 ft., old diggings, Calmalli, Baja California, January to March, 1898 (Herb. Univ. Calif., 2 sheets; Herb. Field Mus.; Herb. U.S. Nat.); *Dr. Forrest Shreve* 6869, at 3 miles north of Punta Prieta (N. Lat. 29°), Baja California, Feb. 17, 1935 (type, Herb. Field Mus.); *idem*

6884, at 10 miles west of Bahia de Los Angeles (N. Lat. 29°), Baja
California, Feb. 20, 1935 (Herb. Field Mus.); *idem* 7058, near Santa
Rosalia (N. Lat. 27°25′), Baja California, Mar. 9, 1935 (Herb.
Field Mus.); *idem* 7164, at 28 miles south of Medano Blanco (N.
Lat. 25°15′), Baja California, Mar. 19, 1935 (Herb. Field Mus);
Dr. Street, Baja California (Herb. Gray).

COREOCARPUS SHREVEI **latilobus** var. nov.—Folia principalia usque
ad 8 cm. longa, bipinnatifida, segmentis latioribus ultimis saepe
7–10 mm. latis.

Specimens examined: *Dr. Edward Palmer* 299, Guaymas, State
of Sonora, Mexico, 1887 (type, Herb. U S. Nat.: cotypes, Herb.
Gray; Herb. N.Y. Bot. Gard.; Herb. Univ. Calif.).

Coreocarpus robustior sp. nov.—Herba annua, ∓5 dm. alta,
subglabra, valde erecto-ramosa. Folia subsessilia vel petiolata petio-
lis usque ad 3 cm. longis, petiolo adjecto usque ad 7 cm. longa, 1–2-
pinnatifida, segmentis oblongis vel ovatis vel terminali lineari-
oblongo, mucronulatis, membranaceis. Capitula tenuiter peduncu-
lata pedunculis 2-10 cm. longis saepe 1- vel 2-bracteatis plerumque
circ. 3-congregatis, ad anthesin ∓1.7 cm. lata et 6–8 mm. alta.
Involucrum campanulatum, glabratum, bracteis exterioribus ovatis
circ. 4 mm. longis. Flores ligulati ∓5, tubo glabro circ. 1 mm. longa,
ligula oblonga vel anguste obovata ∓6 mm. longi, albi vel praecipue
secundum ∓4 venas rosacei vel subpurpurei, apice vix denticulati
Paleae anguste vel late lineares, ∓4 mm. longae. Flores disci sul-
phurei, circ. 2.5 mm. longi. Achaenia exteriora corpore ipso 3.5–4
mm. longa et 1 3–1.8 mm. lata, alata alis sub 0.5 mm. latis nunc
pectinatim incisis nunc separatim in dentes cuneatos divisis, apice
saepe biaristata aristis minutis retrorsum hispidis.

Specimens examined: *G. N. Collins, T. H. Kearney*, and *J H.
Kempton* 214, Agua Verde Bay, Baja California, Apr. 3, 1931 (type,
Herb. U.S. Nat.).

Differs from *C. parthenioides* Benth. in its greater height, erect-
branching habit, much larger and differently shaped leaves, mostly
longer peduncles, etc. From *C. shrevei* var. *latilobus*, to which it
bears a resemblance in its leaves, it differs in its more robust habit,
coarser involucres, larger achenes, etc.

CURRENT LITERATURE

Temperature and Living Matter (Protoplasma-Monographien, 8th vol.). By JAN
 BĚLEHRÁDEK. Berlin: Gebrüder Borntraeger, 1935. Pp. x+277. figs. 70.

The scope of this volume is indicated by the titles of the chapters, which are
as follows: General principles of biological temperature action; rate of biological
processes at biokinetic temperatures; variation of temperature coefficients with
external and internal factors; theories of temperature coefficients; chemical
properties of living systems at biokinetic temperatures; variations of morpho-
logical equilibria at biokinetic temperatures; physical properties of living sys-
tems at biokinetic temperatures; freezing and frost resistance; chilling; chill-
coma and death by chilling; injury by heat and heat resistance; and stimulative
effects of temperature. The presentation of the animal material is somewhat
more effective than is that of the plant material. One finds omissions of material
that would have allowed a somewhat more balanced presentation. However,
the work is a very satisfactory monograph. The general principles discussed are
applicable to plant protoplasm even when the attention is centered mainly on
animal behavior. The previous monographs by KANITZ (1915) and PRZIBRAM
(1923) are superseded by this work of BĚLEHRÁDEK. The advances made during
the last 10 years have been significant and valuable. The author index of 37
pages indicates the widespread interest among investigators in the effects of
temperature upon organisms. The book will repay careful reading. It is written
in English, which will be especially appreciated by American students.—C. A.
SHULL.

Structure and Reproduction of the Algae. Vol. I. By F. E. FRITSCH. New York:
 Macmillan & Co., 1935. Pp. xvii+791.

The first of two volumes on the structure and reproduction of the algae has
recently appeared. These are designed to present a comprehensive account of
the morphology of the algae in the English language, a task for which the
author is well fitted through his lifelong work with this group of plants. But it
is no easy task which he has assumed on account of the diversity of forms found
in the group and because of the divergence of opinion among investigators as to
relationships and taxonomic treatment.

The work is designed as an introduction to the study of the algae in the
widest sense, and with this purpose in mind the author has included all the
holophytic Protista, as well as their non-holophytic allies. The citation of litera-
ture is very extensive and includes practically every paper of significance from
1890 through 1933. The citations are included at the end of each order treated.

The algae have been divided into eleven classes as follows: Chlorophyceae (Isokontae), Xanthophyceae (Heterokontae), Chrysophyceae, Bacillariophyceae (Diatoms), Cryptophyceae, Dinophyceae (Peridinieae), Chloromonadineae, Euglenineae, Phaeophyceae, Rhodophyceae, and Myxophyceae (Cyanophyceae). The term "phyceae" has been adopted wherever the class includes forms with an algal organization, while for those forms in which none is known the old flagellate designation is retained.

Volume I includes a general introduction and the first eight classes as listed in the preceding paragraph. In the introduction are discussed such topics as algae and Flagellata, the broad classification of the algae, the range of structure, the special features of the algal cell, and the general course of reproduction among the algae. The classes are treated by orders, according to their phylogenetic origins, and the orders are arranged by families, with practically all the known genera included.

For the most part the illustrations are judiciously selected from the works of various investigators, and clearly reproduced.

It can scarcely be hoped that all algologists will agree with the arrangement followed in this work, but the presentation of such a vast amount of information in such compass will be stimulating and helpful not only to those whose major interest lies in this field but also to those whose interest is more or less casual.—J. M. BEAL.

A Flower Book for the Pocket. By MACGREGOR SKENE. London: Humphrey Milford, Oxford University Press, 1935. Pp. 380. $3.00.

Often one is requested to recommend a general, reasonably simple book, readily fitting the pocket, that will aid in the identification of wild flowers. Such a book is now available for the identification of British plants. More than 800 species are given, many of them illustrated by line drawings or in full color. The illustrations are good, but one could wish that those in color were somewhat more definite and true to natural tints and shades. The keys are simple, clear, and the descriptions have been kept non-technical without sacrifice of accuracy.

A book, or more probably several books, of similar style, for the flora of the United States should prove a satisfaction to both amateur and professional botanists in America.—E. J. KRAUS.

Primitive Land Plants. By F. O. BOWER. London: Macmillan & Co., 1935. Pp. xiv+658. Illus. 449. $8.00.

BOWER, who is well known as an authority on the Archegoniatae, has recently published a book on the primitive land plants. Since the appearance of his Origin of a Land Flora there have been great advances in facts relating both to the living Archegoniate plants and to the fossil forms. The Rhynie plants serve to link the bryophytes with the pteridophytes, but the gap between the algae and the bryophytes remains as great today as ever. The present book is divided into

two parts. The first part includes 23 chapters presenting the known facts of the several classes of the Archegoniate forms, from the Anthocerotales through the Filicales. Free reference is made to such works as VON GOEBEL's Organography, CAMPBELL's Mosses and Ferns, and to BOWER's Origin of a Land Flora and The Ferns. A comparison and summary are given at the close of the larger groups. The second part includes six chapters given to the discussion of features common to all the classes, such as alternation, embryogeny, and the spore producing members, etc. A chapter is included on the general organographic analysis, and finally a summary of results and conclusions. This work is not a new edition of Origin of a Land Flora, but is an altogether new book. Alternation of generations as demonstrated by HOFMEISTER for the "higher Cryptogamia" formed the foundation for this study of the Archegoniatae. The primary source of the material was the living Archegoniate plants compared with the facts of paleontology. The Archegoniatae are treated as an independent, evolutionary innovation on land, rather than a direct extension of the thallophytes. The "inward urge" toward increase in size, with its accompanying complexity of structure, may take effect in both the gametophyte and the sporophyte generations. The dominant haploid phase of the bryophytes is compared with the dominant diploid phase of the pteridophytes with its telomes, enations, roots, and organs of intermediate character. This work will be a source of inspiration to all who are interested in the more primitive plants from an anatomical, physiological, or organographic standpoint.—C. L. DEEVERS.

Handbuch der Systematischen Botanik. By R. WETTSTEIN. Leipzig and Vienna: Franz Deutticke, 1935. Pp. x+615. Illus. 354.

The second and final volume of the fourth edition of WETTSTEIN's Handbuch der Systematischen Botanik, the first volume of which has been noticed already in these pages (BOT. GAZ. 95:175. 1933), has recently appeared. Continuing the detailed series of specialized treatments of the Anthophyta begun in the first volume, this second volume treats the entire group of Angiospermae. Seventy-eight pages are given principally to prefatory discussions of the Angiospermae as a group and of their major subgroups. These pages deal mainly with phylogeny, relation of floral structures and methods of fructification to those of the Gymnospermae, and leading points of view regarding the systematic arrangement of the Angiospermae. They conclude with a synoptical tabulation of the more important subdivisions.

The individual families are abundantly illustrated with both drawings and photographs, the 354 *Abbildungen* containing a total of no fewer than 2083 figures, all of a high standard of excellence. Bibliographies are full and comprehensive. The typography seems as flawless as it is humanly possible to secure. Students who use the *Handbuch* for serious work will perhaps be impressed most, however, by the evidences of high scholarship and intelligent conservatism manifest throughout its pages. For these we are indebted not only to the late Dr. RICHARD WETTSTEIN, who was the principal author of the earlier editions

of this monumental work, but also to his son, Dr. FRITZ WETTSTEIN, who took the partially finished manuscript after his father's death and brought it to completion.—E. E. SHERFF.

Gymnosperms, Structure and Evolution. By C. J. CHAMBERLAIN. Chicago: University of Chicago Press, 1935. Pp. xi+484. figs. 397.

This recent volume is not merely a revision of the Morphology of Gymnosperms by COULTER and CHAMBERLAIN, for it is completely reorganized and rewritten. This is indicated by the illustrations alone, more than a third of them being entirely new. Fully three-fourths of the illustrations, many of which were used in his previous publications, were prepared by the author himself. CHAMBERLAIN'S drawings are always executed with superb skill and they set a high standard in the technique of botanical illustration. Through personal investigation, the author has contributed to the morphology of nearly all of the groups of gymnosperms, so that he writes with the authority of one who is thoroughly familiar with a wide range of material and who has devoted a lifetime to teaching and research in the morphology of this group of plants.

The subject is organized and treated in twenty-one chapters, of which five are devoted to the living cycads, six to the conifers, three to Gnetales, one each to the Ginkgoales, and to the fossil Cycadofilicales, Bennettitales, and Cordaitales, with chapters on phylogeny and on the alternation of generations.

The gymnosperms are plants of extreme age, reaching back at least 300 million years. The earliest records in the Paleozoic show that there are already two distinct lines included in this group, Cycadophytes and Coniferophytes. The Cycadophytes are traced back to the Cycadofilicales, the Coniferophytes to the Cordaitales. These groups in turn lead back as distinct and separate lines to the Devonian.

The Bennettitales and Cycadales were both derived independently from the Cycadofilicales, not the latter from the former, as many have supposed. Likewise the Coniferophytes include two lines, Ginkgoales and Coniferales, derived more or less independently from the Cordaitales which became extinct at the close of the Permian. Both of the latter are represented by living forms, while the Cycadophytes are represented only by the Cycadales. The Gnetales appear to be much more recent and their origin is more obscure, although some of them show a definite affinity to the Coniferales.

The chapter on Cycadofilicales presents an excellent structural interpretation of this paleobotanical material. Many of the illustrations are new and take into account the excellent restoration of a Carboniferous landscape now among the permanent exhibits of the Field Museum of Natural History. The author also presents a series of hypothetical reconstructions with diagrammatic figures of the steps in the evolution of a seed, including the seeds of the Cycadofilicales. A seed is defined as a megasporangium which retains its megaspore. Here the reviewer would wish to raise the question of whether this definition is really adequate, or whether it should not include the continued nourishment of the female

gametophyte and its embryo through the megaspore wall. However that may be, CHAMBERLAIN takes issue with SCOTT and SEWARD, who have expressed doubt (on the basis of lack of paleobotanical evidence) as to the derivation of heterosporous forms from homosporous ancestors. From a lifetime of study of the comparative morphology of living plants, especially pteridophytes and gymnosperms, CHAMBERLAIN believes that the course of evolution has been from homospory through heterospory to the seed, and ably defends this thesis. The genetic line must have been homosporous Filicales, heterosporous Filicales, Cycadofilicales.

Five chapters are devoted to the Cycadales, the group which has been CHAMBERLAIN's special field of investigation. He has collected and observed them throughout the world in their native habitats. Among the features which are new are more detailed accounts than have been found in his earlier books of the gametophytes of *Microcycas* and of stem anatomy, also the addition of taxonomic keys to the genera, and a brief account of experiments on hybridization among cycads.

The Coniferales are treated in six chapters. Here we find much new material, including phases of general and developmental anatomy less adequately covered in the earlier books on gymnosperms. Considerable research which has been accomplished in recent years in the embryogeny of conifers has been incorporated.

The Gnetales are treated in three chapters in which the author brings together the results of many new investigations. Even in this group, the author's treatment shows evidence of considerable original research. While he has drawn freely upon the accounts of STRASBURGER, HOOKER, PEARSON, and others, he shows many new photographs and figures, including such new details as the floral development of both the male and female flowers of *Welwitschia*.

In his chapter on phylogeny, CHAMBERLAIN summarizes some of his ideas of plant evolution alluded to throughout the book, including observations and conclusions gained from his exhaustive studies during many years. Many readers might wish to have this chapter treated more fully as one feels that the author could enlarge in many places. "It seems safe to say that from the Carboniferous onward the two great lines, Cycadophytes and Coniferophytes, have been distinct. They have some common characters, but the general outline of the life-history is the same in all seed plants, and the pteridophyte structures from which the seed evolved are similar, even in lycopods and ferns. If the two great lines of gymnosperms had a common origin, it is still to be demonstrated."

In the final chapter, alternation of generations is discussed more as it appears throughout the plant kingdom rather than in the gymnosperms alone.

A complete bibliography of 719 titles is a useful feature of the book. The style of the text is excellent and is well adapted as a textbook for advanced courses. Not only because of its review and reorganization of the subject, but also because of the valuable contribution of its many new details, this book is an indispensable reference for the investigator in this field.—J. T. BUCHHOLZ.

THE

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STUDIES IN CELLULAR PATHOLOGY

I. EFFECTS OF CANE GALL BACTERIA UPON GALL
TISSUE CELLS OF THE BLACK RASPBERRY

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 464

W. M. BANFIELD

(WITH PLATE I AND ONE FIGURE)

Introduction

It is the purpose of this paper (1) to present procedures and criteria by which the bacteria which induce and are present in cane galls of black raspberry (*Rubus occidentalis* L.) may be differentiated from all bodies which occur naturally within the cells of this plant; (2) to describe the locus and migration of the parasite within the tissues of the galls which it incites; and (3) to describe some of the effects of the organism upon the cells of this plant. Throughout this research the major emphasis was placed upon critical comparison of the cytoplasms of healthy and pathic cells and upon the differentiation of bacteria from the bodies of like form and size which occur in the cytoplasm of this plant.

Cane gall is a bacterial disease of bramble fruits which occurs widely in the United States and which has been reported from Europe (104, 105). On the black raspberry in the Great Lakes area the bacterium incites numerous white, soft, parenchymatous galls upon the surfaces of the aerial portions of the fruiting canes during the fruiting season. It has been assumed by many pathologists that this disease is induced by the crown gall organism *Phytomonas tume-*

faciens (*Bacterium tumefaciens* Smith and Town) Bergey *et al.* Con-
clusive proof of the identity of the cane gall and crown gall diseases
of bramble fruits is wanting. Available evidence by BANFIELD (2, 3)
and PINCKARD (82) suggests that the organisms inducing these
diseases are distinct.

Materials and methods

The material studied has been of three types: (a) galls of various
ages found occurring naturally on black raspberry and collected from
plantations in the Great Lakes area; (b) galls induced on black rasp-
berry plants in the greenhouse by inoculation with pure cultures of
the cane gall bacterium; and (c) root tip and apical stem meristems,
for purposes of comparison, from plants free from this disease. Com-
parative study of fresh and fixed tissue was made in all phases of the
research.

The tissues to be studied in the fresh condition were collected im-
mediately before use from plants in the greenhouse. At all times
during preparation or examination they were kept immersed in
sterile sucrose solutions of 5–10 per cent concentration or in sterile
water. Sucrose solutions of 7.5 per cent concentration induced no
conspicuous changes in the uncut cells of fresh sections during ob-
servation periods limited to a few hours, whereas 5 or 10 per cent
solutions induced appreciable changes. Cells mounted in sterile
water quickly degenerated. Sections were prepared freehand from tis-
sue fragments held in pith soaked in the appropriate mounting fluid.

Sections were mounted under thin covers on glass slides which
were placed on the stage of the microscope in oil immersion contact
with a Zeiss aplanatic condenser (n.a. 1.4). A Zeiss appochromatic
water immersion objective (70×n.a. 1.25) with compensating oculars
(10, 15, or 20) was used for studying the finer cellular detail. Light
of sufficient intensity was obtained from a 4.5 ampere carbon arc,
the rays of which were condensed by a 3-liter Florence flask which
was placed between the arc and the microscope and which con-
tained cupra ammonia solution of desired color intensity. A second
filter of blue ground glass was placed immediately under the con-
denser of the microscope.

Various fixation and staining methods were used for more perma-

nent mounts. The standard methods used in the study of mito-
chondria were found to give the best fixation of the bacteria in the
tissues of diseased plants. Among these the fluids of REGAUD IV b
(52, 51, 86), NEMEC (78, 68), ERLICKI (52, 51), MOTTIER's modifica-
tion of BENDA (71), and the following BENDA-ERLICKI technique
gave the best fixation of the bacteria.

BENDA-ERLICKI FIXATION

SOLUTION A.---Chromic acid, 1 per cent.............   17 cc.
              Osmic acid, 2 per cent..............   3 cc.
              Acetic acid, glacial................. add 2 drops
SOLUTION B.—Copper sulphate, 2 per cent..........   1 volume
              Potassium dichromate, 5 per cent.....   1 volume
              Mix just before using.

Fix 36 hours in solution A, wash two hours, fix 24 hours in solution
B, wash 24 hours in running water, dehydrate and imbed.

These same fluids, particularly the REGAUD, faithfully preserved
the structure of the cytoplasm as seen in fresh uncut cells mounted
in isotonic sucrose solution, and were found adequate for the study
of the effects of the bacteria upon the structure of the cytoplasm.
Haidenhain's iron-alum haematoxylin gave intense and sharply
selective staining of the bacteria and chondriome.[1]

This stain was used almost exclusively in this work. The selective
staining of bacteria is discussed in a later section.

I. Differentiation of bacteria from cell contents

A. NON-INFECTED TISSUES

a. Microchemical tests

Microchemical tests were made to ascertain the nature of three
classes of ergastic bodies usually encountered in abundance in cer-
tain cells of plants affected or unaffected by the cane gall bacterium,
and in the cells of galls induced by the cane gall or crown gall organ-
ism. These three classes of bodies were: (a) yellow globular and
granular tannin bodies precipitated by the fixing fluids employed,
(b) starch grains, and (c) fat globules. Because of their similarity in

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[1] A term introduced by GUILLERMOND (32) to designate mitochondria and plastids
collectively.

size and form these bodies at times might be confused with bacteria, but generally they are characteristically different. Since these three classes of bodies appeared to account for all cellular inclusions usually encountered aside from mitochondria, vacuoles, plastids, or bacteria, microchemical tests other than those deemed necessary to establish the nature of these bodies were not made.

1. TANNIN.—Pale yellowish to brown granules and globules of various size are present in abundance in many of the cells of material fixed in the fluids used for preserving mitochondria by the REGAUD, BENDA, or NEMEC techniques respectively. These bodies may be dark brown or black as a result of the action of osmic acid after fixation in BENDA's fluid. They may be stained black after immersion in haematoxylin and differentiation for mitochondria, when the tissue is fixed in any of the fluids used in this work for the preservation of the chondriome. In size they vary from granules 1–5 μ in diameter to single globular structures which may occupy practically all the space within the walls of particular cells. Granules may be imbedded within such globules. Unlike the other ergastic inclusions discussed, tannin appears to occur more abundantly in the vacuole of the black raspberry cell.

Tannin bodies occur chiefly in the ray parenchyma cells of the phloem and pericycle, and in the cortical cells of the black raspberry stem. Often most of the cells in certain areas are filled with these bodies. They are found in like abundance in many of the cells of certain areas of gall tissue in which cell division does not occur and in which the cells appear to have differentiated. Such bodies have not been observed in the cells of unstained fresh sections. They appear, however, as globules of various dimension within the same classes of cells in which they occur in fixed material in sections of fresh tissue that have been immersed in 8 per cent formaldehyde solution, or in iodine potassium iodide solution. They appear as globules and granules in sections of fresh tissue treated with 1 per cent chromic acid or potassium dichromate solution or in the fixing fluids of BENDA, NEMEC, REGAUD, or ERLICKI. These granules and globules stain light to dark blue black in 10 per cent ferrous sulphate, green to greenish black in 10 per cent ferric chloride, and turn brown or blacken when in contact with osmic acid vapor. These are reac-

tions characteristic of tannin (101) and these bodies are therefore regarded as tannin bodies precipitated by the fixing fluids employed.

When dilute solutions of neutral red are added to the mounting medium, numerous globules within the cytoplasm of certain cells appear deep red or violet. A granular precipitate of like color can be seen in many large vacuoles and a diffuse red color without precipitates occurs in others. Large globules which absorb the red color also are seen in other vacuoles. It appears that these globules and granules which stain in living cells immersed in dilute solutions of neutral red are similar if not identical to those globules and granules which are rendered visible in fresh cells by bathing the cells in iodine or formaldehyde solutions, and which stain with ferric salts, or blacken in osmic vapor. These color responses have been attributed to the presence within the vacuole of phenolic compounds of the tannin group by the DANGEARDS (16, 19), GUILLIERMOND (32–42), and DUFRENOY (21–28). KLERCKER (50), LLOYD (59, 60), MICHEL-DURAND (30, 31), McNAIR (67), and GUILLIERMOND (41) report tannin as commonly occurring in the vacuoles of certain cells of various plants. Tannin bearing and non-tannin bearing vacuoles may exist side by side in the same cell (50).

2. STARCH.—The relatively large (2–5 μ) spheroidal or irregularly rounded bodies of high refractivity which are abundant at times in the living cells of sections of root tip or gall tissue impart a decidedly granular appearance to these cells, and have the following properties: (a) double refraction to polarized light, (b) digestibility in diastase, and (c) deep blue black staining with iodine potassium iodide solution. These bodies range in size from 0.4 to 5 μ. Frequently their refractivity is only slightly greater than that of mitochondria. Similar bodies but of low refractivity give no blue black color when treated with iodine solution although they may give a slight violet color with iodine solution after treatment in chloral hydrate. Because of these properties they are considered to be plastids which contain a varying quantity of starch as indicated by their refractivity and size.

The tissues of some galls are rich in starch grains, meristematic and degenerating areas excepted. In the early stages of development, galls usually contain very little starch. The ray, pith collen-

chyma, pericycle, cortex, and phloem parenchyma cells of first
year canes are rich in starch grains. Only a few scattered cells of
the pith contain starch grains and then in but small numbers.

3. FAT.—Small spherical bodies of high refractivity are char-
acteristically present in the cytoplasm. If fresh sections of root tip
or gall tissue are treated with Sudan III as described by TUNMANN-
ROSENTHALER (101), numerous bright red globules ranging in size
from 0.2 to 2.0 μ appear in the cells. If these refractive globules are
observed under the microscope as a solution of Sudan III in 70 per
cent alcohol is introduced under the coverslip, they can be observed
gradually absorbing the dye. Sections treated in alkanin react simi-
larly. The bodies in question have an olive cast when in focus, and
their shadow when out of focus is markedly intensified after exposure
to osmic acid fumes, which causes the globules to appear dark brown
or black. When seen in the vacuole or in water they have diffraction
rings or halos about them, but this halo does not appear when they
are in the cytoplasmic ground substance. After treating fresh sec-
tions of root tip or gall tissue with ether or chloroform, neither these
minute highly refractive bodies nor mitochondria can be seen, nor
can they be stained in the cells of such tissue by osmic acid vapor,
Sudan III, or alkanin. Because of these reactions these minute
spherical bodies of higher refractivity than the mitochondria are
assumed to be of fatty nature. They have been described by DAN-
GEARD (16, 17, 18) and by GUILLIERMOND (34, 36, 37, 39, 40, 42) as
being common constituents of the cytoplasm of most if not all
plant cells. They have been variously termed microsomes, sphero-
somes, cytosomes, lipoid granules, fat globules, etc.

b. Cells of root tip

Since cane galls are largely composed of meristematic cells, the
apical meristem of root tip or stem was used for comparative studies
on healthy tissues. Root tips were used more extensively because
they are readily and abundantly obtained by layering the tips of
first year canes during midsummer to late autumn, and because
they, like gall cells generally, are devoid of chloroplasts.

Living cells of root tips as observed in fresh sections mounted in
7.5 per cent sucrose solution are characterized by the following gen-

eral features. The cell walls appear as luminous sharp linear bands of appreciable thickness, usually as parallel pairs with a faint dark line, the middle lamella, between. The cytoplasm of the cell appears as a translucent opalescent fluid substance without visible structure (figs. 1, 2 of pl. I), closely appressed to the cell wall and bearing within it variously shaped transparent areas, the vacuoles. The interfaces of these and the cytoplasm (in other words, the so-called vacuolar membranes, **65, 66, 49, 34**) appear as thin, linear, delicate, bright bands of light. Ordinarily the vacuoles of these cells contain no visible bodies. The cytoplasmic layers between the vacuoles and the cell walls or between the vacuoles are later referred to as cytoplasmic channels.

The cytoplasm of root tip cells contains a wealth of variously shaped small bodies of diverse refractivity. The more conspicuous and the largest are irregularly rounded, usually spheroidal, starch bearing plastids. These bodies move very slowly if at all in the cytoplasmic stream, and tend to collect into groups over the nucleus, or at various other points in the cell. Spherical aggregations of starch bearing plastids are common in root cap cells. Constantly moving over and around these plastids or aggregations of plastids in the cytoplasmic stream are much smaller (0.3–0.4 μ) spherical bodies of high refractivity, the fat globules.

The nucleus usually appears as a perfectly regular sphere or spheroid in the interior of which the nucleolus appears as a spherical, faintly yellowish, finely granular, opaque structure resembling a plastid. In the cells of the apical meristem the nucleolus is large (4.0–7.0 μ) and occupies most of the nuclear volume, whereas in the elongated cells of the plerome or of the periblem the nucleolus is much smaller (1.0–1.5 μ). The nucleus shifts in the cytoplasmic stream, but this is usually perceptible only when comparative observations of its position are made over a period of hours.

Very faintly luminous bodies of diverse form move continuously across and about the nucleus, the clear vacuoles, and the aggregations of starch bearing plastids. They may be spherical structures of the same or slightly smaller diameter than the fat globules, rods, or long filaments, or threads of corresponding diameter and from 2 to 12 μ in length. These are the mitochondria. They are the most

abundant bodies and likewise the most difficult to observe within the cells of this plant.

Since this research has been directed primarily toward an analysis of the pathic state among cells influenced by a bacterial parasite, most attention has been given to those structures within the cells of living and fixed tissue which simulate the form and motion of bacteria. As the latter in many instances (53, 90, 43, 44) have been shown to undergo changes in morphology during their life cycle, and appear as rods, cocci, and smaller particles, all similar forms in healthy cells have been studied critically.

1. MITOCHONDRIA AND FAT GLOBULES.—In the cytoplasmic channels many tiny spherical, rod shaped, or threadlike bodies of low refractivity move amid numerous spherical forms of approximately like diameter but of much greater refractivity. During any brief period of time all these bodies usually move at a fairly uniform speed in one direction past any given point in such a channel. Within the elongated cells of the root tip, which have but thin layers of cytoplasm between the cell walls and the large central vacuole, the motion of these bodies may be clockwise about the cells when viewed in that focal plane that presents the illusion of being the cross section of the cell. It is more complex in such cells as that shown in figure 1, where connecting strands of cytoplasm traverse the vacuoles.

In such cells the bodies may be seen to come from various directions along these strands to a common point near the surface of the nucleus, where after remaining still or swaying about for a few seconds they depart serially along the same course. Along the top or bottom of the cell lumen these minute bodies of the cytoplasm often seem to follow no regular course, but swaying about for a few seconds, may suddenly move in an even regular fashion off to the wall of the cell and disappear downward from the focal field as though impelled by some definite current. Concentrating at a point, they often may move singly or in small groups off to the side and from the side across the top of the cell and down. The motion of these bodies presents every evidence of being that common to bodies themselves without motility but impelled by currents in the cytoplasmic ground substance, or hyaloplasm. The fat globules may move at the same speed as the mitochondria but generally they move

at an appreciably greater speed. The motion of these bodies indicates that there is cytoplasmic streaming in the cell. Without such ready points of reference it might not be detected.

The number of bodies included in the clear cytoplasmic ground substance is very great in root cap cells or in the elongating or unelongated cells of the periblem or plerome. The entire cytoplasm seems to be filled with them, at times scarcely leaving a place large enough for one such body unoccupied. Usually all move at a uniform speed within a stream that travels around the cell over and around the nucleus, vacuoles, and clumped masses of plastids. Within this stream each moves separately, carried by the hyaloplasm, threads bend at either end or in the middle, rod jostles over rod or spherule, so that the cytoplasm appears filled with moving bodies of various size and form. LEWITSKY (56) and MAXIMOV (64) describe a like condition for *Elodea* and *Cucurbita* hair cells.

Mitochondria vary in form in the cells of root tips. Within cells of the root cap or elongating cells of the plerome their most prominent form is a thread. Spherical forms of the same diameter are generally present with the threads, and rod forms of like diameter are plentiful. In the region of elongation of the periblem spherical and short rod forms are more common; threads are scarce. Spherical forms were the only forms seen in the chondriome of the cells of the apical meristem in fresh tissue mounts.[2] Although the mitochondria of these various cell groups generally have the form indicated, this form is decidedly not constant. Thread forms may be abundant in elongating cells of the periblem. At times thread forms may not be present in any of the root tip cells but only rod and minute spherical forms may occur. While thread and rod forms may predominate in cells of the root cap at the beginning of an observation, only minute spherical forms may be visible some hours later.

Root tip cells fixed and stained by the methods of REGAUD, BENDA, or NEMEC in general reveal the same forms repeatedly observed in fresh tissue cells. In fixed root tips, however, cells of the root cap generally are devoid of thread forms even though in other regions the elements of the chondriome as seen in fresh cells may

[2] In none of the fresh material examined was the detail of the chondriome adequately clear in these particular cells, owing perhaps to the density of the cytoplasm.

be differentiated clearly. Moreover in fixed material the cells of the apical meristem frequently contain numerous thread forms, whereas in fresh cells only spherical or short rod forms have been seen. It is also evident from repeated study of such fixed and stained material, mounted in water and observed with the water immersion objective of relatively high magnifying power, that many elements of the chondriome remain unstained and are readily overlooked if at all visible when such sections are mounted in balsam. For these reasons the observations made upon fresh tissue sections are considered to be more critical in many respects than those made on material fixed, stained, and mounted in balsam after the standard methods generally employed in research upon the chondriome.

2. PLASTIDS.—Cells of root tips in addition to mitochondria and fat globules contain another order of cytoplasmic inclusions which are generally of greater size and of varying form. These are the plastids. When devoid of starch they are of the same order of refractivity as the mitochondria, and are as difficult to see. They are spherical when seen in fresh tissue mounts of apical meristem cells. In fixed tissues they appear as rodlike or spherical forms in the cells of this region. In the elongating cells of the periblem they are spherical, rod shaped, or of bacteroid form (fig. 1). They vary upward in size from that of mitochondria. Farther back from the root tip they become larger, are generally spherical, and attain a maximum size of 5 μ. In the elongating cells of the plerome their form varies from threadlike to rodlike, or from bacteroid to spherical forms distended by starch inclusions (fig. 2). Until they bulge at one or either end or increase evenly throughout their length, they may not be distinguished from thread or rod shaped mitochondria.

Dumb-bell, hand-mirror, or thread forms with bleblike swellings occur in cells of both plerome and periblem in the region of elongation, but are more common in the former. Farther back from the root tip all these bodies are spherical or irregularly rounded and from 2.0 to 5.0 μ in diameter. In the cells of the root cap they appear spherical. In such cells they are usually very abundant, are generally grouped in motionless clusters, and appear to be chiefly of one size in any particular cell (2–5 μ in diameter) although there are

usually present less conspicuous forms intermediate in size between these and mitochondria.

The refractivity, mobility, and distribution of these bodies within the cytoplasm of the cells of root tips vary with the differentiation of these cells. In the cells of the apical meristem their refractivity is as that of mitochondria; they are evenly distributed in the cytoplasm; and neither they nor mitochondria have been seen to move. In the elongating cells of the periblem and plerome, their refractivity is slightly greater than that of mitochondria and they are evenly distributed throughout the cytoplasm. The motion of the larger ones, however, is considerably slower than that of mitochondria. Because of their large size and the high refractivity of the inclosed starch, they are the most conspicuous elements of the cytoplasm in the elongated cells of periblem and plerome and in the cells of the root cap. Their motion is not apparent although slight changes of position may be noted over a period of hours. Within such cells they tend to aggregate in clumps along the wall, and particularly over the nucleus. This clumping is characteristically pronounced in the cells of the root cap. The abundance, large size, and high refractivity of these starch bearing plastids at times impart to these cells a pronounced granular effect. Although these bodies may be of equal size and be present in equal numbers in the cells of other root tips, they are inconspicuous because of their low refractivity, which may be only slightly greater than that of the mitochondria. Plastids develop from structures which are indistinguishable from mitochondria in the cells of the apical meristems of root and stem of the black raspberry (fig. 3). This is the condition commonly reported for seed plants (91, 32, 34, 39, 92, 71, 102, 85, 107).

Changes in osmotic properties of the mounting medium may markedly alter the cell. The most pronounced visible effects are changes in mobility of the cytoplasm, volume and structure of the vacuolar system, and the form of mitochondria and plastids. Motion of fat globules varies considerably with the state of hydration of the cytoplasm. The motion of the cytoplasmic bodies is regular within the cytoplasmic stream in cells of fresh sections that are mounted for a short time in distilled water. Their brownian motion is faintly if at all perceptible at first, but changes rapidly as the cell imbibes

water. The large vacuoles within the cell may coalesce; if a single vacuole is present it distends, forming an even thinner layer of cytoplasm against the wall. The nucleus usually is seen in this layer as a flattened bulge against the wall. The mitochondria and plastids become irregularly swollen, diminish in visibility, and finally disappear from the cell. As they swell they aggregate in chains or clusters much as starch filled plastids do, and lose their mobility. The fat globules which moved so deliberately begin to oscillate in wider and ever wider arcs, until the cytoplasm seems a mass of wildly dancing particles, upon which no clear focus can be obtained. Now all regular streaming in the cytoplasm has ceased, the motion of the minute fat globules dancing chaotically becomes slower and slower, then finally stops entirely. At this time the vacuolar boundaries change. Up to this time they have been clear cut, gracefully rounded lines, marking the interface of the two fluids, cytoplasm and vacuole. Now, as though some inner pressure of the vacuole had been suddenly released, these lines become irregularly curved and wavy; the vacuole has collapsed. Within a few minutes the sharp line of demarcation disappears and the limits of the cytoplasm may be only inferred from the non-moving minute fat globules which the cytoplasmic ground substance contains. A few granular particles, perhaps precipitates of cyclic compounds formerly present in solution in the vacuolar fluid, are now seen, together with occasional starch grains in the vacuolar cavity in brownian motion. If a 7–10 per cent sucrose solution be substituted for the distilled water, the cells do not recover the appearance which cells of freshly cut sections have when mounted in isotonic sucrose solution. Changes in the osmotic pressure of mounting medium are of no avail. With the last faintly discernible motion of the cytoplasmic bodies vibrating *in situ*, the changes effected by anaesthesia (46) have become irreversible, and perhaps death has come at this moment to the cell. Hence, after 5 to 8 hours, no motion save that in collapsed vacuoles may be seen in cells of sections of tissue mounted in distilled water.

The same transition in type of motion of the cytoplasm is seen when toxic vital dyes such as Janus green B[3] are added to the

[3] The writer is indebted to Professor BLOOM of the department of anatomy, University of Chicago, for a sample of the highly purified Janus green B used by Dr. BENSLEY in his pioneer work with this dye.

mounting medium. The transition occurs within a few minutes at dilution 1–10,000 of Janus green B, but extends through several hours when the cells are immersed in distilled water.

All attempts to stain mitochondria in the streaming cytoplasm of living galls failed with solutions of Janus green B. The dilutions used varied from 1–10,000 to 1–2,000,000. The mitochondria remained unstained although clearly discernible in the streaming cytoplasm of cells that had been immersed 12 hours in dilution 1–2,000,-000, Janus green B. Some of the mitochondria (rods, spheres, threads) were stained in the cells after the cells had died. Those stained were generally clumped in small irregular masses scattered throughout the rigid fixed (12) hyaloplasm. Janus green B, therefore, was not a useful vital stain that could be used effectively in this research. Similar results have been reported by others who have studied plant cells (15, 85, 107, 1).

B. GALL TISSUE CELLS

In the early stages of gall formation on the canes of black raspberry the actively dividing cells usually are regularly oriented, as though all divisions occurred tangentially to the radial axis of the cane. As the galls increase in size and age, tracheids appear irregularly distributed throughout the gall tissue; the cells in certain regions become heavily charged with tannin as do the ray cells in the stem; differentiation of most of the cells stops at the parenchyma level with various degrees of maturation indicated by decreasing cytoplasmic content. At the base of the gall new cells may continue to arise for a time, until cell production in this area of the gall ceases.[4]

Within the gall tissue, however, scattered whorls of densely cytoplasmic cells are evidence of continued cellular division within certain limited areas randomly distributed. The older galls are therefore aggregations of fairly regularly oriented, parenchymatous cells of varying age, and appear approximately uniform in size, equivalent to parenchyma cells of pericycle or phloem in canes not affected by the bacterium.

WULFF (104) and BUTLER (11) expressed the view that the gall is

[4] The base of the gall is here considered to be that innermost layer of gall cells in contact with cells of the stem which have not conspicuously changed in response to the bacterial invasion.

the resultant chiefly of pericyclic activity. From observations and a limited number of experiments it appears that the organism migrates upward from the crown of the plant through the tissues of the stem. At what time and through what tissue this migration occurs is not known at present.

The mitochondria of gall tissue cells (fig. 4) display no conspicuous changes that cannot be found in cells of unaffected plants. They usually are evenly distributed in the cytoplasm, and the motion induced by cytoplasmic streaming is the same as that in the cells of root tips. The mitochondria are present as short rods, spheres, or threads in the youngest cells of embryonic regions in gall tissue. Enlarging cells which are rich in cytoplasm and have multiple or single vacuoles usually have numerous thread forms. This is the condition of mitochondria in most gall cells. The number of mitochondrial filaments or rods is large in those cells which are rich in cytoplasm, and appears to be reduced in those gall cells which have thin sheaths of cytoplasm between their voluminous central vacuole and the cell walls. This condition is not appreciably if at all different from that found in the various cells of plants free of the disease. MILOVIDOV'S (70) observations on crown galls of *Pelargonium* and *Vicia faba* led him to similar conclusions with respect to the mitochondria in gall tissue cells.

Plastids in living gall tissue are diverse in size, form, and refractivity. In younger cells cylindrical forms with rounded ends are as abundant as spherical ones. Such rods measure 2–7 μ by 0.3–1.6 μ. The smaller forms, rods, threads, or spheres are of low refractivity, are moved about in the cytoplasmic currents, and usually are evenly distributed within the cytoplasm of the cell. The large rods and spheroidal forms with a diameter that exceeds 2 μ are shifted about very slowly, if at all, and tend to aggregate in groups along the wall in the peripheral cytoplasm and especially over the nucleus. The bacteroid form (that is, the rod form with a bulge or two along its length) occurs fairly frequently. These plastids are the most conspicuous bodies and may occupy most of the volume of the cytoplasm in older cells, particularly in galls induced on plants in the greenhouse in late autumn or early winter. Their large size, abundance, and high refractivity due to large volumes of included starch make them conspicuous.

Fat globules are present in all living gall cells. In the younger cells they are slightly larger and more refractive than the spherical mitochondria. In older cells their size and number increase markedly. This is also true of the ray parenchyma and corresponding cells of the stem. The fat globules usually are evenly distributed throughout the cytoplasm of these cells. In fixed preparations they may stain black with haematoxylin if they are preserved. Usually they are removed in the process of dehydration and clearing.

It is apparent from these observations that the mass of gall tissue is initially composed of cells in every respect comparable with analogous cells of meristematic regions of plants unaffected by cane gall. There is little evidence that they are pathic. Later changes occur in these cells, either as a result of subsequent direct or indirect action by the bacteria or lack of healthy correlation (57, 58) with the rest of the plant. The locus and movement of the bacteria in the gall tissue are first described, however, since these degenerative changes of gall tissue cells are conditioned by the locus and movement of the bacteria within the tissue. Before these changes are taken up, the differentiation of bacteria, plastids, and mitochondria in cane gall tissue will be discussed.

C. Micro isolation and staining

Only spherical bodies of high refractivity and of varying diameter are visible in the cells of fresh sections of cane gall tissue mounted for some time in hypotonic solution such as water. These bodies move incessantly in the cytoplasm of the living cells. The nuclei and vacuoles may be clearly discernible in the cells of such sections, as may also starch grains if present; but aside from these bodies the cells appear to be devoid of structure. The spherical bodies seen usually are 0.2–0.5 μ in diameter and have all the properties and give all the microchemical reactions characteristic of fat globules. As a result of diminished refractivity coincident with characteristic swelling in hypotonic solution, the mitochondria and non-starch bearing plastids are not visible within the cells of gall sections mounted in this way.

Cane gall bacteria, invariably present in gall tissue, are readily differentiated from the elements of the cytoplasm under such conditions. The individual thalli, uniformly short rods with rounded

ends, usually are seen in masses. Their refractivity generally is slightly lower or equal to that of fat globules (fig. 5). They appear opalescent to faintly yellowish, their surface membranes appear as perfectly regular delicate lines, and within the thalli there is no visible structure under the conditions and with the equipment named. The individual rods constituting these masses generally are regularly arranged end to end in parallel rows. They are motionless and at times appear to be imbedded in a slimy sheath. Those thalli which are seen within degenerate cells generally appear as single or bifurcated rods, slowly oscillating in brownian motion in the cell fluid.

Since mitochondria and plastids of varying size and form are visible within thin sections of fresh gall tissue mounted in isotonic sucrose solution, and since many of these units of the chondriome of the black raspberry under these conditions are like bacteria in form, size, and refractivity (fig. 4), the identity and differentiation of the bacteria from these elements of the chondriome is difficult; the picture is confusing.

Experimentally some evidence has been obtained by micro dissection as to the non-bacterial nature of these rod shaped bodies of bacterial proportion seen in living cells of gall tissue when mounted in isotonic sucrose solution. Using an aseptic technique throughout, these bodies were removed with micro pipettes and placed singly or in numbers within drops of sterile media suitable for the growth of bacteria. Coverslips bearing the hanging drops were sealed with vaseline over depressions in deep well slides containing approximately one-tenth of 1 cc. of the same medium as that in the hanging drop. These drops were incubated six days at 26° C., and were then discarded if no growth appeared. Those which produced growth were plated and the colonies which appeared were inoculated into black raspberry. The ability to induce cane galls subsequent to inoculation on one-year-old black raspberry canes was the criterion used for determining the presence of pathogenic bacterial colonies. A CHAMBERS' micromanipulator (13) and the technique described by WRIGHT and McCOY (103) were used.

Seventeen of 21 attempted cultures of these cytoplasmic rods were negative, and from four, yellow colonies developed. As a check

on the effectiveness of this method, 23 attempts were made to iso-
late and culture the masses of bacterial rods found in pockets within
gall tissue. Thirteen of these cultures produced no growth, eight
produced cultures of yellow Schizomycetes, and two produced cul-
tures of cane gall bacteria. Because the bacteria were successfully
cultured in only 9 per cent of the isolations made from bacterial
pockets, the negative results obtained in culturing the rod shaped
bodies of bacterial proportion isolated from living gall cells are
considered merely suggestive, not convincing.

The appearance which these rods have in culture fluids when iso-
lated from the cytoplasm of gall cells is not characteristic of bac-
terial rods. The latter, when isolated from the bacterial masses of
the gall tissue, habitually have a perfectly regular clear-cut outline,
and a homogeneous opalescent interior which is without visible
structure. The rods taken from the cytoplasm of living gall cells,
when isolated in yeast broth culture medium (88) containing 1 per
cent of glucose, had a pronounced granular appearance and were
neither as regular nor as clear-cut in outline as bacterial rods[5] isolated
in like manner from bacterial cavities in the gall tissue, or as these
same cytoplasmic rods when observed in the cytoplasm of living
cells.

A detailed study was made of fixing and staining methods to find
a procedure or combination of procedures whereby bacteria might
be positively differentiated from the elements of the cytoplasm with
which they are easily confused. In preparations of cane gall tissue
fixed in ERLICKI's hardening fluid and stained in haematoxylin,
plastids and bacteria usually are stained alike a brilliant deep blue,
in striking contrast to the orange cell walls and faintly gray cyto-
plasm. Fat globules and mitochondria retain the stain poorly if at
all, and appear light gray or are not visible. The form and size of
the bacteria aggregated in the usual masses or strands are constant.
The plastids are of varying size and form and are scattered through-
out the cytoplasm or aggregated in chainlike or other groups at
various points within the cytoplasm. In many cells their dominant

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[5] These rods observed in isolated drops of culture fluid after removal from the cyto-
plasm probably were rodlike granules of starch remaining after spontaneous degenera-
tion of the isolated plastids.

form is rodlike and in size and staining they are the exact counter-
parts of bacteria. Only an occasional transparent unstained starch
granule within some of these rods, as well as their apparent identity
to like forms seen in cells of healthy root tips, indicate their plastid
nature.

Tissue fixed in the fluids of BENDA, REGAUD, NEMEC, or ERLICKI
and stained with haematoxylin may give good fixation but no
differentiation of bacteria and plastids. Cane gall tissue fixed in
NEMEC's second fluid or in certain variations of the BENDA fluids,
and stained in Haidenhain's iron-alum haematoxylin, at times shows
excellent fixation and brilliant elective staining of the bacteria. The
mitochondria and plastids may be readily destained, leaving an in-
tense blue only in the bacteria. Plastids and bacteria retain the stain
in like degree in other fixations with these same fluids. Differentia-
tion between rodlike plastids and bacteria is therefore not possible
with this stain, and the results are confusing. Because of gross dis-
tortion in form and size of mitochondria, plastids, and bacteria, other
fixations with the NEMEC or REGAUD fluids may be worthless. When
used alone, therefore, this method might lead to the conclusion that
an intracellular parasite characterizes gall tissue.

Excellent differentiation of the bacteria from all elements of the
cytoplasm in cells may be secured by fixing the tissue in BENDA's
chromic osmic fluid, hardening in ERLICKI's fluid, and staining in
haematoxylin. Bacteria, plastids, and mitochondria are well fixed.
The mitochondria and plastids may be readily destained to a faint
gray, however, leaving an intense blue only in the bacteria. The
plastids and bacteria, which may be alike in form, are differentiated
sharply by the faint gray of the former and the intense blue of the
latter.

II. The parasite and its relation to host cells

A. IDENTIFICATION OF BACTERIA OBSERVED IN
CANE GALL TISSUE

Since most bacteria probably would stain alike if present in cane
gall tissue, and since bacteria other than the causal organism might
bring about some of the cellular effects observed, particularly the
disintegration of the tissue later described, it was considered nec-

essary to establish the identity of the bacterium associated with the cellular and tissue changes noted in the galls. Isolations were made from many galls of various ages induced by pure culture inoculation of plants in the greenhouse or garden, or following natural infection on black raspberry plants in Michigan or Indiana plantations. Fragments of gall tissue, approximately 6–8 cubic mm. in volume, were removed aseptically from various portions within the surface or from the surface of the galls,[6] and transferred to sterile petri dishes containing 2 cc. of sterile water.

The bits of tissue were then macerated, loop dilutions were made to other plates, and from these in turn loop dilutions were made to a third set of petri dishes. All plates were poured with 2 per cent potato dextrose or 1 per cent yeast glucose agar and incubated for ten days at room temperature. Usually 100 or more colonies were obtained, and of one kind, when the isolation was made from the interior or surface of galls that were not more than 25 days old. This type of colony appeared in the potato dextrose poured plates after the fifth day, and after several additional days of incubation was a convex white butyrous small colony 1.5–3.0 mm. in diameter. Its ability to induce cane galls when inoculated into black raspberry was taken as criterion for identification as the cane gall bacterium. Older galls usually but not always yielded increasing numbers of rapidly growing colonies of yellow Schizomycetes and a decrease in number of colonies of cane gall bacteria. Plates poured from the tissue of galls more than two months in age generally yielded only yellow colonies. However, in view of the enormously greater number of colonies of cane gall bacteria isolated in the plates poured from all galls which externally were free of decay, and from gall tissue which microscopically showed the characteristically marked internal tissue degeneration described later, it was inferred that the bacteria isolated from the tissue are the incitants of the cellular changes which characterize cane gall.

That all question as to the identity of the bacteria observed in the tissue might be removed, galls were induced under aseptic con-

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[6] The surface of a gall is taken to be that layer of living or dead cells in contact with the environment. Cane galls have in no case been found to have any organized group of cells comparable with a periderm or a root cap.

ditions within glass cylinders, using the technique described by
BANFIELD (4). Three weeks after inoculation, fresh white frosty
overgrowths were present within these cylinders. On first-year black
raspberry canes they extended 0.5 cm. radially from the stem at the
points of inoculation. The cylinders were filled with sterile water
for ten minutes prior to the removal of the galls for microscopic
study. This water was diluted 1–1000 with sterile water, 1 cc. of the
dilution was placed in each of several petri dishes, the dishes were
poured with 2 per cent potato dextrose agar, and incubated at 24° C.
for seven days. Only the cane gall bacterium appeared in the plates.
The number of bacteria present per cc. of wash water was 48,300 as
determined by the plate count. Cane gall bacteria were the only
organisms that appeared in agar poured plates in which sections of
these galls were macerated.

Microscopic examination of the tissue of these galls, living or fixed,
revealed that they differed in no important way from galls induced
underground or aerially in the greenhouse, field, or garden.

B. LOCUS AND MIGRATION OF BACTERIA IN TISSUES OF CANE GALLS

The bacteria migrate through the tissues in zoogleal strands which
separate the cellulose walls of gall tissue cells by dissolution of the
middle lamellae. Their grouping in chains or in masses is character-
istic; they do not occur singly. At the tips of the advancing strands
there frequently is only a single bacterial rod between the parting
cell walls. More commonly the advancing tip is lobed and contains
a number of bacteria. Behind such lobate tips the bacteria generally
are regularly oriented end to end and evenly spaced, forming a
strand or column of multiple parallel rows. The advancing tip of the
strand may be decidedly broad, the inclosed bacteria irregularly
oriented, and spaced in the tissues of galls which are subjected to a
shower for twelve or more hours prior to fixation of the tissue.

Bacterial pockets commonly occur in the meristematic areas of
cane gall tissue. They result from successive lysis and collapse of
cells in contact with bacterial strands, accompanied by increase in
the mass of bacteria. The cells in contact with the expanding bac-
terial mass are lysed and irregularly compressed inward, thereby im-

parting an irregular outline to the bacterial mass. These pockets become numerous as the galls increase in age.

Extensive intercellular penetration by the bacteria may induce maceration and complete lysis of areas of cells, thereby creating cavities of considerable magnitude in the tissue. The areas affected are chiefly those cells in older galls which are at a distance from the bacterial pockets and outside the meristematic zones that encompass bacterial pockets. Individual cells or groups of cells in such areas may be separated from the tissue by complete dissolution of the lamellae and be engulfed by the bacterial mass.

Within the cells of these areas, swarms of bacterial rods are frequently seen oscillating in brownian motion. These protoplasts are degenerate, however, and only fragments such as fat globules remain within their partially hydrolyzed walls. Occasionally small compact groups of bacteria have been observed within protoplasts which show no pronounced disorganization of cytoplasm or nucleus. Usually, however, penetration of the cell walls occurs only after the protoplast has been extensively lysed and disorganized. Cane gall bacteria are therefore primarily intercellular.

Cane gall bacteria found in strands and masses in gall tissue commonly are inclosed in a gelatinous matrix which in fresh tissue appears as a translucent clear material in which the bacteria are without motion. This imbedding matrix is stained a clear faint yellow in tissue fixed in the fluids of NEMEC, BENDA, REGAUD, or ERLICKI, and stained with haematoxylin and orange G. The bacterial rods in sharp contrast are stained an intense blue. This matrix usually is viscous as determined with micro dissection needles, and individual rods imbedded in it are not readily removable. The bacteria of these masses frequently have been observed in brownian motion in other sections of fresh tissue. They are then easily removable by micro pipettes from these masses. Under such conditions it is obvious that the viscosity of the imbedding material of the so-called zoogleum has been markedly reduced and varies but little from that of the dilute sucrose solution of the mounting medium.

The bacteria appear to be discharged from the tunnels and pockets in which they exist in the gall tissue on to the surface of the gall prior to its disintegration. Similar observations on fire blight

cankers have been reported by NIXON (80). This discharge occurs
after liquefaction of the gelatinous material in which the bacteria
are imbedded. Evidence of discharge is of four types: (1) many
cavities or pockets obviously created by the bacteria are found in
cane gall tissue, but at the time of observation contain few or no
bacteria; (2) bacteria are always found on the surface of both cane
and crown galls of black raspberry; (3) experimental evidence of
the continuous escape of the bacteria from crown galls has been
presented by ROBINSON and WALKDEN (89) and by BANFIELD (4);
and (4) the bacteria have been observed in the process of discharge
via intercellular channels from cavities included in sections of fresh
cane gall tissue.

Discharge of cane gall bacteria from interior pockets via inter-
cellular channels has been observed on several occasions in sections
mounted in 7.5 per cent sucrose solution. This discharge is illustrated
in text figure 1. In a cavity below the sectioning plane (GH) a
faintly discernible shifting of bacteria occurs within a dense mass of
independently motionless bacterial rods, in zone A accompanied
by a very slow motion oriented toward a focal point B. At a point
midway between B and C this shift becomes distinct and the bacteria
are oriented end to end as sketched. This orientation and the ac-
celeration in motion that follows are much like that which occurs
to driftwood in the smooth water immediately above a rapid in a
river. From C to F the motion of the rods is extremely rapid but
decidedly jerky, as though instantaneous stopping of the inter-
cellular channel of discharge occurred (as at point D), followed by
momentary release, stopping and release, etc., causing the bacteria
to move at high velocity in spasmodic jerks. The general picture is
similar to the discharge of spores in certain Oomycetes (20). The
bacteria are discharged from the opening of the intercellular space
at the surface of the tissue F (the surface formed by the plane of
sectioning). The velocity of the bacterial rods is quickly lost in the
zone F to X, and beyond X the motion is that of slow diffusion
radially away from the point of discharge. The bacteria, now float-
ing free in the mounting medium bathing the tissue, oscillate in
brownian motion but otherwise their motion is not appreciable.
They may become extremely numerous in the immediate area about
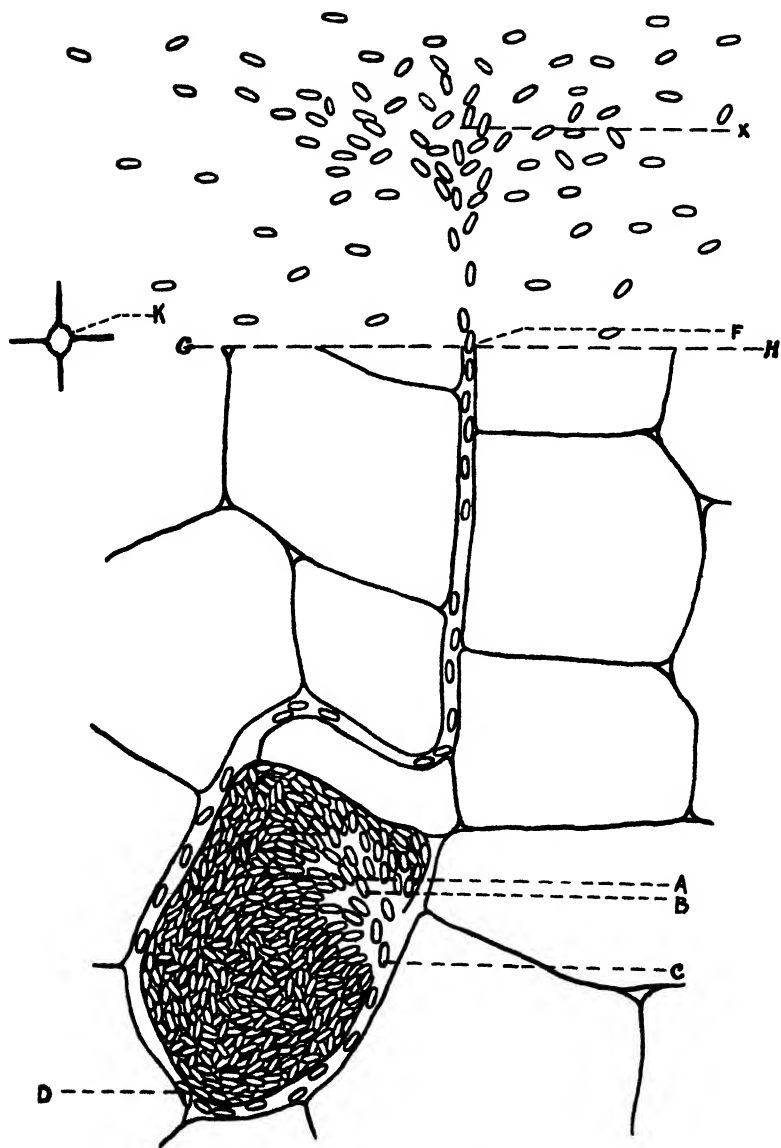
TEXT FIG. 1.—Discharge of cane gall bacteria as seen under the microscope from an interior cavity within a fragment of gall tissue. The bacteria are being discharged via an intercellular space formerly occupied by a zoogleum into the mounting medium (7.5% sucrose).

the point of discharge, F, but each remains free and there is no tendency to agglutinate into masses as is exhibited by the elements of the chondriome in autolyzing cells.

The channel through which this discharge occurs appears in cross section as at K in the sketch. Invaginations of the normally distended cell walls are characteristic of these channels of escape. From this, and from the like contour of cross sections of intercellular spaces occupied by bacterial strands, it appears that these channels are passages which were occupied by bacterial strands whose contained thalli have been discharged.

C. Effects of bacteria upon host tissues and cells

The gall development which the cane gall organism incites on fruiting black raspberry canes appears to be essentially wound response. The abundant cellular division by which the gall tissue is formed occurs primarily in those regions which are at a distance from the bacteria.

Hyperplasia of the pericycle and phloem ray cells induces the gall initials or bulges found beneath the phelloderm of fruiting canes in early spring.[7]

The first series of divisions leads to the formation of a sheath of cells which at first envelopes the initial foci of infection. Into this sheath the bacteria subsequently penetrate radially from the initial foci of infection. The cells surrounding these strands in turn are stimulated to division, the cross walls of the new cells being laid down parallel to the bacterial strands or masses, the source of stimulus. Lateral penetration among these cells by bacterial strands may occur and again be followed by division of the cells surrounding the penetrating strands. As the galls increase in size and age, however, hyperplasia becomes progressively less.

Cellular degeneration and necrosis are coincident with hyperplasia from the time of the first microscopic appearance of the gall in early spring. The cells in contact with the bacteria gradually undergo cytolysis, those cells beyond divide and divide, while those at a farther distance apparently are not stimulated. In the early stages

[7] Hypertrophy of cells, a common response of many plant cells to tissue invasion by the crown gall organism (97), does not occur in this disease on black raspberry.

of gall development, however, new cells are produced so rapidly and in such profusion that the lytic action of the bacteria on the relatively limited number of cells with which they are in contact appears insignificant. The division of cells becomes progressively more feeble, however, the bacteria more and more numerous, intercellular invasion more extensive, and ultimately tissue degeneration becomes general.

This degeneration is the result of two types of cellular disorganization and disintegration: (1) *Cytolysis* is a degeneration characterized by the progressive lysis of the plastids, mitochondria, nucleus, fat, and eventually the walls of protoplasts in contact with bacteria. The fat volume of protoplasts undergoing this type of degeneration is greatly increased. (2) *Autolysis* is a degeneration characterized initially by changes in the condition and aggregation of the chondriome to form various irregular solid masses, alveolar, granular, reticular, open three dimensional network, or perforated plate structures in the cytoplasm. In this degeneration all elements of the cell eventually lose their identity and are blended in an amorphous mass of diminishing volume, compressed between collapsing walls. It usually is accompanied by no appreciable changes in the fat volume of the protoplast. It occurs most conspicuously in masses or large areas of cells enveloped by or separated from the rest of the tissue by sheaths of cytolyzed cells and bacteria.

In the degeneration of the tissue large numbers of cells disintegrate as a result of cytolysis. The great majority of cells degenerate through autolysis, however, probably as a result of lack of healthy correlation with or complete isolation from the rest of the tissue. These degeneration phenomena are apparent upon casual microscopic observation of the gall. The direct effects of cytolysis are more conspicuous during or for a short time after periods of rain in the blossoming period of the black raspberry. At such times larger areas of the outer tissue of the white rounded gall masses become butyrous to the touch; the tissues offer no resistance to slight pressure and readily rub away. Extensive intercellular invasion of bacteria and dissolution of lamellae have macerated these areas of cells. A few days later the galls appear extensively pitted. They undergo no conspicuous changes in color as a result of this type of

degeneration, and remain white or have a slight cream color and a water soaked appearance. The effects of autolysis are more apparent later. The gall tissue turns yellowish, then brown, and shrivels. Galls induced by inoculation undergo like changes.

The progressive lysis of cells in contact with bacteria generally appears to proceed in the following manner. Starch is rarely present in such cells. The plastids are reduced to elongated or threadlike forms which after a time, if still present, are indistinguishable from the small rodlike and spherical mitochondria (fig. 5). Mitochondrial rod and thread forms are reduced in number, while the small spherical forms increase. Fat becomes conspicuously more abundant in the cells.[8]

The nucleus is reduced in size and after a time displays a faintly granular or reticular internal structure. Streaming of the cytoplasm ceases, for a time the cytoplasmic bodies vibrate in brownian motion, then all motion ceases. The mitochondria disappear, fat globules and the nucleus remain (fig. 6). The granular structure of the nucleus is accentuated, and the nucleolus is reduced in size proportionate to the reduction in volume of the nucleus. The outline of the nucleus becomes irregular and finally the nucleus disappears. Fat is usually the last substance to disappear from such cells. Many cells are reduced in this way, leaving merely their walls. Along the borders of bacterial pockets or strands, the walls of these cytolyzed cells collapse and are mingled as débris among the bacterial mass. It has not been possible, however, to follow any one cell through this entire sequence of changes.

The cellulose walls of these cells in the majority of instances are not dissolved by the bacteria. They may retain their usual position during or subsequent to the lysis of the protoplast; they may be slightly compressed by the expanding mass of bacteria; or they may collapse entirely after complete lysis of the protoplast, so that their inner surfaces touch. Small areas of the wall may be dissolved by bacterial masses which then may occupy the entire cell lumen. In

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[8] This rise in fat volume is common in cellular degeneration induced on a wide range of plant and animal cells by fungi and bacteria (21–29), exposure to radium emanations, x-rays or ultraviolet light (72, 75, 76, 27), various lipoid solvents (6, 7, 73, 74), heat (54, 84), osmotic changes (34, 8, 9), poisons (94), inanition (79).

areas of tissue maceration, however, the lytic action of the bacteria may include complete lysis of the walls of many cells and partial lysis of cells at the periphery of the area of completed lysis. The end result may then be an empty irregular gap in the tissue after the bacteria have been released from the area. Occasionally advancing bacterial strands appear to pass through cellulose walls but as yet no convincing demonstration of this has been observed.

Usually cytolysis occurs relatively slowly and is observable more commonly in cells which have but a portion of their wall in contact with the bacteria, although it may occur in those areas macerated by the bacteria. In the latter instance it may be effected in like manner but much more rapidly. Usually within the cells of areas extensively penetrated by bacteria autolytic changes occur before cytolysis is completed, and the tissue of these areas degenerates as a result of both types of cellular disintegration. There is a pronounced rise in fat volume in these cells coincident with extensive bacterial penetration, and the plastids generally are reduced in size or disappear (fig. 5). At this stage the remaining elements of the chondriome may fuse into a network (fig. 8), reticulum (figs. 7, 8), or agglutinated mass (fig. 9). The vacuoles and nuclei in such cases appear relatively unchanged. In more pronounced degeneration of this type the vacuoles disappear and all elements of the protoplast lose their identity in an amorphous mass of débris occupying a small space within the collapsing walls.

Mitochondria, plastids, and fat move freely about over and around one another in the streaming cytoplasm of non-pathic cells (fig. 4). As the cells undergo autolysis, spatial isolation of these bodies generally is lost. The elongated plastids and mitochondria of such cells may display a pronounced tendency to adhere wherever they touch each other. For a time this union may be transitory, and the mitochondria join, break away, then join with others. The ends of the same mitochondrial filament may fuse, producing a ring, or several may fuse in various ways, the result being a small open network. These rings and small networks may for a time be carried about in the cytoplasmic stream. Other mitochondrial filaments touch and fuse, the process continues, and the network enlarges by addition of more and more of the free mitochondria of the cell. Free

filaments coming into contact with each other or with the network
of filaments are held. The filament may then break away, move about
in the cytoplasm, touch another filament and again be held momen-
tarily or permanently. Eventually all the mitochondria may become
fused into this network. The network may be in one plane but gen-
erally is three dimensional, and often is generally distributed
throughout the major area occupied by the cell (fig. 8).

Small bacteria have frequently been seen to move through such cut
cells under and over the filaments of the network in water mounts of
fixed sections observed with the water immersion objective. This
would not be possible in a *multiple* vacuolate condition such as that
implied repeatedly by DUFRENOY (21–29; in particular 21, pls.
IIIa, VI, VII).

Whether production of the network structure proceeds from start
to finish in this fashion or is subsequent to and a progressive
stage of the plastid reticular fusion elsewhere described is not clear.
The type of fusion here described is common to cells wherein
fusion of the chondriome has already attained considerable propor-
tion and the free mitochondrial filaments are conspicuously reduced
in number. Free spatially isolated non-starch bearing plastids, such
as are numerous in non-pathic cells, generally are not present in these
cells. This absence of plastids, and the observation that the plastid
reticulum may transform into a network structure, suggest that
reticular fusion may be an initial stage in the formation of network
structures. The fusion of limited numbers of mitochondria or
plastids has been described repeatedly (6, 7, 69, 42, 28). CHAMBERS
(12) has described the mitochondria of an Orthopteran germ cell
clearly stainable by Janus green B as forming a voluminous granular
network. Fusion of the elements of the chondriome to form net-
works has been reported to occur in tissue culture of chick embryo
heart by LEWIS· and LEWIS (54, figs. 5, 6, 8; 55, fig. D, pl. III).
DUFRENOY (21, 22) and BEAUVERIE (7) have reported an increase
in the mitochondrial complement of various types of plant cells
prior to their disintegration.

There appear to be currents in the cytoplasm during the forma-
tion of the network. These currents carry the mitochondrial fila-
ments from place to place prior to their more permanent fusion.

Doubtless this movement of the cytoplasmic ground substance in-
duces the continuous swaying of the filaments of the reticulum or
network for a time, even after all mitochondria of the cell have be-
come fused into the network. Fat globules also move about in cells
in which mitochondrial networks are forming. They may lie free
and vibrate in brownian motion between the swaying network
strands. Frequently they appear to adhere to the strands before or
after the mobility of the cytoplasm has been lost.

The mitochondrial network may undergo condensation or it may
break up into a granular débris. Smaller condensed masses of heavier
network strands and plate structure of high refractivity are fre-
quently observed in cells wherein all cytoplasmic bodies have lost
their identity. Mitochondrial networks have been observed to con-
tract during the course of observation, and by a continuation of the
fusion process to give rise to similar but condensed and knotted
networks, perforated plate, or massive strand structures. The latter
are decidedly vacuolate in appearance at times owing to open areas
in the original network which become partially inclosed by mem-
brane-like sheaths formed by lateral fusion of mitochondria and
plastid filaments. Condensation of plastids and mitochondria where-
in no initial network is formed produces somewhat similar masses of
material of heightened visibility (fig. 9).

Fragmentation of mitochondrial networks has been observed. It
occurred gradually in networks in fresh tissue that was studied over
a period of hours and suddenly when distilled water was added to
the sucrose solution in which the sections were mounted. In the first
instance networks were clearly visible in numerous cells of a particu-
lar microscopic field during the two hours immediately following sec-
tioning and mounting of the tissue. The networks observed in two
cells of this field were sketched. Four hours after mounting, con-
densation had occurred in the network of one of these cells. The
strands of the other were of markedly diminished visibility, and at
the end of the following hour only granular particles and clear-cut
fat globules could be seen. The network had apparently dis-
integrated. The addition of distilled water to the sucrose mounting
fluid bathing another section of fresh tissue caused the swaying net-
work in several cells to break up within a few moments to granular

débris in which only a few mitochondrial rings, short rods, and network fragments could be readily identified. Associated with areas of cells in which clear-cut networks abound, there are usually other cells in which mitochondrial fragments (rods, spherical forms, occasional threads with their long axes parallel to each other and closely appressed) lie massed together and irregularly distributed in the cells.

This is readily observable with a water immersion objective in fresh or fixed material (fig. 9). It is difficult to detect in sections fixed, stained, and mounted in balsam because of irregular staining or no staining at all. When stained the individual elements are not resolvable and blend together in an opaque mass, unstained; the difference in refractivity between them and balsam is not great enough to make them visible. PARAT (81) points out that the intense staining or chromatophile area of degenerate cells where strand structure is apparent are areas where mitochondria and vacuoles are in intimate contact and where lipoids are particularly abundant.

The cells in which fragmentation of the network has occurred generally are found nearer to the surface of the gall than are the cells in which no fragmentation has occurred. Only granular débris is found in cells still nearer to the surface or periphery of the gall. Even this granular appearance is lost and nothing remains but a spongy homogeneous mass in collapsed cells on the surface of such areas of the gall. In progressive degeneration of cells observed in fresh and fixed tissue, the granular débris present at certain stages is very probably of mitochondrial and plastid origin.

Mitochondria may not display any tendency to fuse prior to the moment of death. With the cessation of cytoplasmic movement they are held *in situ* in the rigid cytoplasm and remain for a time spatially isolated. Their refractivity is not diminished. Lying in slightly different planes curved about the margins of numerous vacuoles, and overlapping, they may produce symmetrical patterns which may impart a superficial alveolar structure to the cytoplasm. Careful observation, however, reveals this apparent structure to be the result of numerous variously curved mitochondrial threads, isolated from one another in the clear cytoplasmic ground substance by very slight distances.

Radial strand structures are produced in certain areas of degenerating cells. This structure, composed of irregular strands of varying mass and diameter, probably arises by subsequent hydration of the rigid clear cytoplasmic ground substance and fusion of mitochondria and plastids that are spatially isolated at the moment of cellular death. In many of the cells of certain areas studied in fresh sections in which this strand structure was prominent, mitochondria and plastids were observed to be spatially isolated in the rigid hyaloplasm. In adjoining cells mitochondria and plastids were seen to lie close together and were tightly interwoven to form strands (fig. 9). In others the elements that made up this particular type of strand were apparently fused and usually gave no indication as to the origin of the strands. Starch grains are occasionally included in these strands, however, and the position of mitochondria and plastids in adjoining cells indicates that the strands are products of these elements of the cytoplasm. A radial strand structure, the strands of which are perfectly regular in outline and of uniform diameter throughout their respective lengths, may also result from the plastid mitochondrial reticular fusions which occur prior to the moment of cell death.

Mitochondria may display no tendency to fuse before or after death of the cell, and lose their identity with the other cytoplasmic structures in granular débris. A few small spherical or rodlike forms of very low refractivity may still be identified as of mitochondrial nature in such cells in fresh tissue. In material fixed in the fluids of BENDA, ERLICKI, NEMEC, or REGAUD and stained with iron-alum haematoxylin, the granular appearance is accentuated by the uneven staining of the various granular particles, among which occasional rodlike, short thread, or spherical forms may still be readily identified as of a plastid or mitochondrial nature. All cellular elements in such cells lose their identity after a time and are resolved into the doughy compact mass of irregularly folded homogeneous material lying between the collapsing walls of degenerate cells. This material in fresh sections takes a faint red stain with Sudan III and browns somewhat with osmic acid. It stains intensely with haematoxylin, after the fixations enumerated in an earlier section. Granular structure in the cytoplasm is not found in cells wherein the mitochondria

have fused to form various reticular strand, network, or mass structures. These condensation structures do not stain with osmic acid or Sudan III in fresh tissue. The larger strands and masses and certain of the reticuli stain intensely in haematoxylin after fixation as described. The networks and isolated filaments are more difficult to stain, and are apt to stain irregularly or not at all with the usual staining schedule for plastids and mitochondria.

Plastids may aggregate end to end in chains, and by fusion their ends may become resolved into a reticulum in autolyzing cells (fig. 7). Such reticuli and the various stages in their formation have been observed in both fixed and fresh cells. In the initial stages bodies clearly of plastid nature are seen in chainlike aggregations. The limits of the individuals between the ends of the chain later are not resolvable, and the entire chain oscillates slowly as a unit in the cytoplasmic stream. In other cells the reticulum is longer and more mobile. The irregular diameter of this reticulum throughout its length, its refractivity and staining properties, indicate the origin of the reticulum. The progressive decrease in the number of free plastids in the cytoplasm appears to vary directly as the increase in size of this reticulum. Finally few or no free plastids remain. Mitochondrial filaments may fuse into a reticulum or network structure prior to, subsequent to, or simultaneously with the fusion of plastids into a strand structure.

Bodies which appeared to be plastids have been observed in the vacuoles of cells in degenerating areas of gall tissue. These bodies were of characteristic plastid size, form, mobility, and refractivity. In one instance a dumbbell-shaped plastid-like body was observed to be extruded almost instantly from cytoplasmic ground substance into a vacuole. A number of other elongate club-shaped, dumbbell-shaped, and variously rounded irregular rodlike bodies with clear-cut regular outline were already present in the vacuole of this cell. Like bodies of usual plastid form, size, refractivity, and mobility were also present in the cytoplasm of this cell.[9]

In other cells wherein all plastids have disappeared from the

[9] Tannin globules of like and varying diameter are also present in gall cells. They are generally perfectly spherical and, unless treated with intra-vitam dyes or other reagents, are not visible under the conditions of this research.

cytoplasm, long filaments, uniform in diameter throughout their length, have been repeatedly observed in the central vacuoles. These filaments are of characteristic plastid refractivity, are extremely long, and form a profusely recurved reticulum. There may be several shorter ones but usually there is only a single long one in the vacuole. The nuclei of such cells display no conspicuous variation from that observable in the nuclei of non-pathic cells. Mitochondria are clearly visible in usual number and size in the cytoplasm of some of these cells which have reticuli in their vacuoles. Fat is unchanged. In one instance, in a cell in which such a reticulum was observed at the beginning of a three-hour observation period, the reticulum had disappeared from the vacuole at the close of the period. A network structure composed of strands of like diameter was then present at the interface of vacuole and cytoplasmic ground substance.

Plastids display a pronounced tendency to agglomerate and fuse to solid masses in autolyzing cells. The large starch bearing plastids commonly clustered about the nucleus of living cells generally fuse into a dense agglomerate mass in the autolyzing cell. Smaller groups scattered throughout the cytoplasm may also agglomerate and fuse into irregular homogeneous masses of heightened refractivity. The outlines of these masses for a time are studded with the spherical protuberances of but partially fused plastids. Eventually fusion becomes complete, and all traces of the origin of the substance composing the mass are lost as the individuals which make up the mass lose their identity in it. Starch grains are frequently observed in these masses in the initial stages of fusion, but apparently are soon digested since they are not generally found in the compact smooth masses of the end stages of fusion.

Associated with plastid fusion of this type is the mitochondrial fusion already described. Radiating from the agglomerated plastid masses, irregular strands and plates of fusing mitochondria or the strands of mitochondrial networks frequently are seen. Isolated plastids are also found in these strands. Occasionally, even after fusion has progressed to the point of obliterating the origin of these strands, the fact that plastids have formed a part of their substance is disclosed by an occasional starch grain in the strands.

Agglutination of the chondriome to form homogeneous masses of

varying pattern and size in the cytoplasm of degenerate animal
tissue cells has been described by MAYER, RATHERY, and SCHAEFFER
(65, 66), SCOTT (94), CHAMBERS (12); and in plant cells by BEAU-
VERIE (6, 7) and DUFRENOY (21, 28). DUFRENOY attributes the so-
called X bodies or inclusion bodies of degenerate virus cells to this
agglutination and vacuolization of plastids. SHEFFIELD and SMITH
(96, 100, 95) describe similar agglutination phenomena as forming X
bodies in virus cells but they are noncommittal as to the identity of
the agglutinating elements.

   A number of observations suggest that plastids may form part of
the strands of the network seen in certain groups of autolyzing cells.
The spatially isolated rod or elongated plastids, prominent particu-
larly in mature or cytolyzing cells, are conspicuously absent in the
cells wherein the network structure has been completed. The fusion
of plastids into reticuli in which the identity of the substance of the
recurved filaments is lost, and the transformation of this into a net-
work structure (as already described), are also evidences of plastid
inclusion in the substance of the networks described.

   Plastids and mitochondria may undergo vacuolization and give
rise to an aveolar structure in the cytoplasm of autolyzing cells. The
first changes noted in sections of fresh tissue are the cessation of
cytoplasmic streaming and an aggregation of granular material in
the cytoplasm. In the functional cell the mitochondria move freely
in the cytoplasmic stream and are clear-cut in outline. In these cells
they are indistinct, aggregated, and motionless. They appear to be
swollen. The plastids appear irregularly spheroidal, larger than in
non-pathic cells, and give no starch reaction. They are usually ag-
gregated over the nucleus, but may aggregate in smaller groups or lie
singly in the cytoplasm along the walls. Because of the marked re-
duction of visibility of the mitochondria and plastids in such cells,
the more satisfactory observations have been made on material fixed
in ERLICKI's fluid and stained with iron-alum haematoxylin.

   The plastids are stained an intense blue black; the mitochondria
are generally unstained in cells of gall tissue fixed in ERLICKI's fluid
and stained in haematoxylin. In contrast, the swollen plastids and
mitochondria of autolyzing cells in these same preparations are
stained a faint clear blue. The plastids vary in size upward to four

or five times their usual diameter. Mitochondrial threads occasionally present appear irregularly swollen, as is usual with mitochondrial threads in the cut cells of fresh root tip sections. The spherical form, however, aggregated into compact masses, is characteristic of these autolyzing cells. These small spheres, all of approximately the same diameter, may be aggregated in groups apart from plastids or they may be intermingled with plastid groups. The plastids are spatially isolated from each other as are the mitochondria in non-pathic cells. They are aggregated in clumps scattered about the cell, or more generally they are aggregated over the nucleus of autolyzing cells. Swollen plastids and mitochondria may also be aggregated in chains or arranged in strands radiating from the nucleus to the walls, creating symmetrical patterns of beautiful design. In many instances these aggregations of swollen mitochondria and plastids impart a distinctive foam structure to the cytoplasm.[10]

Where degeneration has progressed further, an irregular very faintly staining membrane-like film of foamlike structure, irregularly contracted, remains. Only scattered wisps of the plastid material remain in others. The apparently rapid reduction in the amount of plastid and mitochondrial substance suggests that the marked increase in surface induced by this vacuolization brings about lysis of the chondriome much more rapidly than it is accomplished subsequent to the plastid mitochondrial condensations described elsewhere.

Fat does not become conspicuously augmented in volume in autolyzing cells. This is one of the conspicuous differences between the cytolysis and autolysis described. Fat is usually present in autolyzing cells in the same sized globules and in like frequency as in functional cells. Increase in age of gall or normal stem tissue, as stated elsewhere, is accompanied by increase in the quantity of fat found in the cell. Occasionally in the débris of autolyzing cells wherein all normal structure has been lost a few large fat globules $2-5 \mu$ in diameter are found. These stain red with Sudan III and blacken in osmic acid. Usually no fat globules are detectable in the

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[10] Vacuolization of the chondriome has been reported frequently in degenerate animal and plant tissue cells (5, 34, 35, 39, 42, 6, 7, 9, 73, 74, 22, 24, 26, 29, 69, 93).

doughy or spongy débris that remains between the collapsing walls of disintegrating cells. This material stains intensely with haematoxylin after fixation in the usual mitochondrial fluids. It also takes a faint red stain with Sudan III and browns with osmic acid fumes. This may be indication of the presence of fat in the cellular débris, but like all other elements of the protoplast it apparently loses its individuality and is blended into the amorphous mass that remains between the collapsing walls of disintegrating tissue.

From the foregoing description of the cytoplasm of functional and autolyzing cells of the raspberry, it appears that such structure as is visible in these cells under the compound microscope is the structure imparted to the degenerating cytoplasm by the various states and arrangements of its included bodies, namely, mitochondria, plastids, and fat. The cytoplasmic ground substance in fresh tissue is a translucent viscous fluid, which after fixation in the fluids of BENDA, NEMEC, ERLICKI, or REGAUD has a very finely granular appearance (fig. 3). The reticular, alveolar, radial strand structure, or network structure observed in autolyzing cells in fresh or fixed tissue, is of mitochondrial and plastid material. The coarse granular structure present in the cytoplasm of certain autolyzing cells, or in non-pathic cells that have been treated with ethyl alcohol (coagulation), is also due to distortion and degenerative changes in the mitochondria and plastids of the cytoplasm.

III. The crown gall controversy

The preceding observations have some bearing on the controversy concerning the position of crown gall bacteria in the tissue of its host plants and the structure of cytoplasm.

SMITH in 1912 (97) presented the evidence which led him to believe that the bacterium is an intracellular parasite. He observed numerous granular bodies, many of which were rodlike and seemingly of bacterial proportion, in the cells of gall tissue that had been treated with gold chloride and formaldehyde. In 1920 (98), however, he admitted that these bodies probably were mitochondria. In 1925 (99), after additional evidence had been presented pro and con, he last expressed his views on the subject in an address before the French Academy of Sciences, and said, "La localisation (-inter ou intra cellulaire-) n'est pas connu."

RIKER (87) and also ROBINSON and WALKDEN (89) took issue with SMITH and demonstrated that cell division and multiplication of gall tissue cells took place in sheaths about what apparently were masses of bacteria in crown gall tissues of tomato, tobacco, and sunflower. They concluded therefore that the position of the organism was intercellular. This view was reaffirmed by MAGROU (61, 62, 63) upon the basis of analogous studies on crown galls of *Pelargonium*, and again by BERRIDGE (10) who studied crown galls induced on sweet pea and tomato. More convincing evidence of the correctness of this conclusion was presented by HILL (47), who demonstrated and published unequivocal drawings showing the intercellular position of crown gall bacteria in early stages of infection in tomato.

Exception to the intercellular interpretation of the locus of crown gall bacteria within the tissues of its host plants was taken by PINOY (83) and by NEMEC (77, 78). PINOY reported bacteria-like bodies on the surface and within certain tannin bearing cells of crown galls on *Pelargonium*. NEMEC (78), in a report on cytological investigations of crown galls occurring naturally on plum nursery stock, said, "Es enthalten alle Gewebearten der von mir untersuchten Tumoren innerhalb der Zellen Bakterien oder bakterienähnliche Gebilde." He inferred that these bacteria-like bodies, which he observed within the cells of plum galls that had been fixed in NEMEC's first or second fluids, were crown gall bacteria because of (1) their uniform size, (2) their tendency to collect in bacteria-like groups in the cytoplasm of gall tissue cells, and (3) the specific differences between these bodies and mitochondria as to form, size, and selective staining properties.

On the basis of the studies herein reported, it is concluded that the bacteria-like bodies observed by SMITH and later by PINOY and NEMEC within tissue cells of crown galls were normal elements of the chondriome of the cells and not bacteria as they at one time believed, for the following reasons.

1. The plastids of most seed plants at certain stages in cell maturation are similar to if not identical in size and form with phytopathogenic bacteria. The tendency of plastids in the cells of seed plants to aggregate in bacteria-like groups within the cytoplasm also is general.

2. Equivalence in size, form, and staining reaction of bacteria and

plastids at certain stages of cellular maturation in cane gall and crown gall tissues on the black raspberry has been demonstrated.

3. Plastids commonly are more readily fixed and have a greater affinity for stains (92, 71, 68, 107) than have mitochondria. The destruction of mitochondria and the resistance exhibited by plastids to fluids containing acetic acid have been shown by GUILLIERMOND (32, 34) and COWDRY (14). The writer has found this to be true of plastids in cane gall and crown gall tissue after fixation in fluids containing acetic acid such as the formalin acetic alcohol commonly used by SMITH (97). Selective staining of plastids in cane gall or crown gall tissue on the black raspberry is readily attained after fixation in the fluids of NEMEC, ERLICKI, and frequently after fixation in BENDA's fluid.

4. The bacteria in crown gall tissue of the black raspberry are frequently decidedly limited in number and in many sections of such galls none may be found. The probability of not observing them and of mistaking selectively stained elements of the chondriome for the bacteria is considerable.

5. It has been demonstrated in this research that cane gall bacteria are primarily intercellular and are not found in living cells of gall tissue on black raspberry. On the basis of limited observation this applies also to the tissues of crown galls induced on black raspberry.

STRUCTURE OF CYTOPLASM IN BLACK RASPBERRY CELL

The structure of cytoplasm has long been a subject of considerable controversy. Few of those who have interpreted this structure have made detailed studies of its contained bodies, namely, mitochondria, plastids, and fat globules, however, nor have they properly evaluated the effects of various fixing fluids upon these elements of the cytoplasm. In this way the reticular, filar, granular, and alveolar interpretations of this structure have arisen. GUILLIERMOND (34, 42) has emphasized the importance of a thorough understanding of these visible elements in evaluating cytoplasmic structure. He has shown that the cytoplasm of plant cells is a polyphasic substance composed of a ground substance, transparent and without visible structure in the living state, finely granular after fixation, and containing mito-

chondria, plastids, and fat globules. In the living cell these elements by their more general threadlike form, fat excepted, impart a structure to the cytoplasm that might be interpreted as fibrillar. An alveolar or foam pattern is generally induced by immersion of the cells in hypotonic solution, which incites the elements of the chondriome to swell so as to form a mass of soapbubble-like films. A coarse granular structure in the cytoplasm is generally induced by treating the cells with absolute alcohol, or the fluids of Carnoy, Bouin, and various mixtures containing acetic acid, which cause a partial disintegration of the chondriome.

Observations of the cytoplasm of living and degenerating cells of the black raspberry lead to reaffirmation of the views advanced by GUILLIERMOND relative to its structure. The reticular, filar, granular, and alveolar structures in the cytoplasm of these cells are products of the various states and arrangements of its contained bodies, namely, mitochondria, plastids, and fat. The cytoplasmic ground substance in freshly mounted cells has no visible structure. It has at most a faint, very finely granular appearance after fixation in mitochondrial fluids.

Summary

1. The bacteria which incite cane gall on fruiting canes of black raspberry are found primarily between the cell walls of infected tissue.

2. The bacteria ramify throughout all regions of cane gall tissue in the form of zoogleal strands which dissolve the middle lamellae of the cell walls.

3. Numerous visibly degenerating or necrotic protoplasts may be filled with bacterial thalli in certain areas of the gall tissue, and frequently the site of the protoplast within the walls is completely occupied by a swarm or solid mass of bacteria.

4. The bacteria also may occur in pockets or cavities which may be numerous and which result from lysis of masses of protoplasts and their cell walls.

5. The bacteria may be discharged from the intercellular spaces or cavities prior to disintegration of the gall.

6. Cells with which the bacteria are in contact undergo a more or less rapid cytolysis. In early stages of gall formation those cells at

a distance from the bacteria may divide repeatedly. Subsequently intercellular penetration may occur, and in turn the cells at a distance may be incited to extensive division. Eventually cell division ceases, extensive intercellular penetration by the bacteria continues, and the gall degenerates.

7. This degeneration is the result of two types of cellular disorganization and disintegration, cytolysis and autolysis.

8. During cytolysis the plastids are first reduced in size, are changed in form, and become indistinguishable from mitochondria. The mitochondria diminish in number and finally disappear from the cell. Coincident with lysis of the chondriome and reduction of cytoplasmic volume, the vacuolar volume of the cells increases and fat globules increase markedly in volume until they become the most conspicuous elements of the protoplast. The nucleus becomes granular, irregular in outline, and smaller until it finally disappears. At this stage fat and cell walls remain. Next the fat disappears and finally portions of the entire wall of the protoplast may be dissolved by the bacteria.

9. In autolysis the elements of the chondriome display a pronounced tendency to aggregate, and there is no appreciable increase in fat volume of the protoplast. Plastids and mitochondria generally fuse to form various reticular, solid condensation masses, or open, three dimensional networks of varying pattern. The elements of the chondriome may become vacuolate or they may fragment into granular débris. Ultimately all elements of the protoplast lose their identity in a granular or doughlike disintegrating mass of diminishing volume between collapsing cell walls, which in fixed material is stained intensely and which in fresh tissue is stained a faint red by Sudan III and brown by osmic acid.

10. The visible structure of the cytoplasm of healthy cells of the black raspberry or of those cells in which a pathic state is induced by the cane gall bacterium is the structure imparted to the cytoplasm by the various states and arrangements of its contained bodies, namely, mitochondria, plastids, and fat. In pathic cells this structure may be granular, alveolar, reticular, or it may appear as various three dimensional open network patterns. The visible structure of the cytoplasm of healthy cells such as those of root tips un-

affected by the cane gall bacterium is that of a polyphasic complex composed of a clear ground substance, without visible structure, in ᾽which the various spherical rodlike or threadlike mitochondria and spherical fat globules are dispersed and in which larger starch bearings plastids may also be dispersed or aggregated in various group patterns.

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LITERATURE CITED

1. BAILEY, I. W., The cambium and its derivative tissues. V. A reconnaissance of the vacuome in living cells. Zeitschr. Zellforsch. Mikrosc. Anat. 10:651–682. 1930.

2. BANFIELD, W. M., Black raspberry cane gall. Wisconsin Agr. Exp. Sta. Bull. 410. 109–110. 1930.

3. ——, Cane gall of black raspberry. Phytopath. 20:123–124. 1930.

4. ——, Life history of the crown-gall organism in relation to its pathogenesis on the red raspberry. Jour. Agr. Res. 48:761–787. 1934.

5. BANG, I., and SJOVALL, E., Studien über Chondriosomen unter normalen und pathologischen Bedingungen. Beitr. Path. Anat. Allg. Path. 62:1–70. 1916.

6. BEAUVERIE, J., La résistance plastidaire et mitochondriale et le parasitisme. Compt. Rend. Acad. Sci. Paris 172:1195–1198. 1921.

7. ——, Action du parasite sur la résistance du chondriome-plastidome; sa fragilisation et l'altération de la structure cellulaire. Proc. Internat. Congress Plant Sci. II. 1299–1311. 1926; issued 1929.

8. ——, Sur la valeur des inclusions huileuses ou lipoides des plastes et des mitochondries. Compt. Rend. Soc. Biol. Paris 98:311–312. 1923.

9. ——, Quelques aspects de la dégénérescence des plastes, applications au parasitisme. Rev. Gén. Bot. 40:206–225; 264–276. 1928.

10. BERRIDGE, E. M., Studies in bacteriosis. Ann. Appl. Biol. 17:280–283. 1930.

11. BUTLER, E. J., Some aspects of the morbid anatomy of plants. Ann. Appl. Biol. **17**:175–212. 1930.

12. CHAMBERS, R., Microdissection studies in the germ cell. Science n.s. **41**:290–293. 1915.

13. ———, New micromanipulator and methods for the isolation of a single bacterium and the manipulation of living cells. Jour. Inf. Dis. **31**:334–343. 1922.

14. COWDRY, N. H., A comparison of mitochondria in plant and animal cells. Biol. Bull. **33**:196–228. 1917.

15. ———, Experimental studies on mitochondria in plant cells. Biol. Bull. **39**:188–206. 1920.

16. DANGEARD, P. A., Sur la nature du chondriome et son rôle dans la cellule. Compt. Rend. Acad. Sci. Paris **166**:349–446. 1918.

17. ———, Sur la distinction du chondriome des auteurs en vacuome, plastidome et sphérome. Compt. Rend. Acad. Sci. Paris **169**:1005–1010. 1919.

18. ———, La structure de la cellule végétale dans ses rapports avec la théorie du chondriome. Compt. Rend. Acad. Sci. Paris **173**:120–123. 1921.

19. DANGEARD, PIERRE, Recherches de biologie cellulaire. Evolution du système vacuolaire chez les végétaux. Le Botaniste **15**:1–267. 1923.

20. DRECHSLER, CHAS., Two water molds causing tomato rootlet injury. Jour. Agr. Res. **34**:287–296. 1927.

21. DUFRENOY, J., Introduction à l'étude cytologique des plantes affectées par des maladies à virus. Ann. Épiphytes **14**:163–174. 1928.

22. ———, Observations sur les modifications pathologiques de la forme des vacuoles des cellules végétales. Ann. Épiphytes **14**:227–268. 1928.

23. ———, Influence du pH du milieu de culture sur les constituants cellulaires des orchidées. Rev. Gén. Bot. **44**:153–158. 1932.

24. ———, Maladies à virus du tabac. Phytopath. Zeitschr. **2**:321–339. 1930.

25. ———, The changes induced in the cytoplasmic structure of cells by virus diseases. Proc. Fifth Internat. Bot. Congress. Cambridge. pp. 367–368. 1930.

26. ———, Les modifications pathologiques de la structure des cellules végétales. Ann. Inst. Nat. Agron. Paris. Ser. 2. **23**:1–104. 1930.

27. ———, Modifications pathologiques du métabolisme cellulaire chez les tabacs. Ann. Épiphytes **18**:259–316. 1932.

28. ———, Die Viruskrankheiten. Phytopath. Zeitschr. **5**:85–90. 1932.

29. ———, Death as the result of change of living matter within the plant cell. Science n.s. **78**:494–500. 1933.

30. DURAND, E. MICHEL-, De l'état des tannins dans la cellule végétale. Compt. Rend. Acad. Sci. Paris **178**:586–589. 1924.

31. ———, Recherches physiologiques sur les composés tanniques. Rev. Gén. Bot. **40**:705–734. 1928.

32. GUILLIERMOND, A., Recherches cytologiques sur le mode de formation de

l'amidon et sur les plastes végétaux. Archives d'anat. microsc. 14:309-428. 1912.

33. ——, Sur la métachromatine et les composés phénoliques de la cellule végétale. Compt. Rend. Acad. Sci. Paris 166:958-960. 1918.

34. ——, Observations vitales sur le chondriome des végétaux et recherches sur l'origine des chromoplastides et le mode de formation des pigments xanthophylliens et carotiniens. Rev. Gén. Bot. 31:372-413; 446-508; 532-603; 635-770. 1919.

35. ——, Observations cytologiques sur le cytoplasme d'un *Saprolegnia*. La Cellule 30:357-378. 1920.

36. ——, Sur les microsomes et les formations lipoides de la cellule végétale. Compt. Rend. Acad. Sci. Paris 172:1676-1678. 1921.

37. ——, Recherches sur l'appareil de golgi dans des cellules végétales et sur ses relations avec le vacuome. Archives d'anat. microsc. 23:1-98. 1928.

38. ——, The recent development of our idea of the vacuome of plant cells. Amer. Jour. Bot. 16:1-22. 1929.

39. ——, La structure de la cellule végétale: Les inclusions du cytoplasme et en particulièr les chondriosomes et les plastes. Protoplasma 16:291-337. 1932.

40. ——, La structure des cellules végétales à l'ultramicroscope. Protoplasma 16:454-477. 1932.

41. ——, Recherches cytologiques sur les pigments anthocyaniques et les composés oxyflavoniques. Rev. Gén. Bot. 45:188-210; 258-276; 307-325; 379-404; 455-472; 508-536; 596-619; 683-695. 1933. 46:50-62; 88-127. 1934.

42. GUILLIERMOND, A., MANGENOT, G., and PLANTEFOL, L., Traité de cytologie végétale. Paris. 1933.

43. HADLEY, P., Microbic dissociation. Jour. Inf. Dis. 40:1-312. 1927.

44. HADLEY, P., DELVES, EDNA, and KLIMEX, J., The filtrable forms of bacteria. 1. A filtrable stage in the life history of the shiga dysentery bacillus. Jour. Inf. Dis. 48:1-159. 1931.

45. HARPER, R. A., The structure of protoplasm. Amer. Jour. Bot. 6:273-300. 1919.

46. HEILBRUNN, L. V., The physical effect of anesthetics upon living protoplasm. Biol. Bull. 39:307-315. 1920.

47. HILL, J. BEN, The migration of *Bacterium tumefaciens* in the tissue of tomato plants. Phytopath. 18:553-564 1928.

48. KITE, G. L., Studies on the physical properties of protoplasm. I. The physical properties of the protoplasm of certain animal and plant cells. Amer. Jour. Physiol. 32:146-164. 1913.

49. ——, Studies on the permeability of the internal cytoplasm of animal and plant cells. Amer. Jour. Physiol. 37:282-299. 1915.

50. KLERCKER, J. E. F., Studien über die Gerbstoffvakuolen. Bihang till K. Svenska Vet. Akad. Handl. 13:Afd. III, no. 8. 1888.

51. KRAUSE, R., Enzyklopädie der mikroskopischen Technik. Berlin. 3 Vols. 1927.

52. LEE, B., Microtomist's Vade-mecum. 9th ed. Philadelphia. 1928.

53. LEVINE, M., Morphological changes in *Bacterium tumefaciens*. Science n.s. 62:424. 1925.

54. LEWIS, M. R., and LEWIS, W. H., Mitochondria (and other cytoplasmic structures) in tissue cultures. Amer. Jour. Anat. 17:339–401. 1915.

55. ――――, Behaviour of cells in tissue culture. Section VII, General Cytology. Univ. Chicago Press. 1924.

56. LEWITSKY, G., Vergleichende Untersuchungen über die Chondriosomen in lebenden und fixierten Pflanzenzellen. Ber. Deutsch. Bot. Ges. 29:685–696. 1911.

57. LINK, G. K. K., The rôle of genetics in etiological pathology. Quart. Rev. Biol. 7:127–171. 1932.

58. ――――, Etiological phytopathology. Phytopath. 23:843–862. 1933.

59. LLOYD, F. E., The tannin-colloid complexes in the fruit of the persimmon, *Diospyros*. Biochem. Bull. 1:7–41. 1912.

60. ――――, The occurrence and functions of tannin in the living cell. Trans. Roy. Soc. Canada 3rd Série. 16: Sect. V. 1–14. 1922.

61. MAGROU, J., Le *Bacterium tumefaciens* dans les tissus du cancer des plantes. Compt. Rend. Acad. Sci. Paris 183:804–806. 1926.

62. ――――, Recherches anatomiques et bactériologiques sur le cancer des plantes. Ann. Inst. Pasteur 41:785–801. 1927.

63. ――――, Le *Bacterium tumefaciens* dans les tissus du cancer des plantes. Inst. Pasteur Bull. 25:563–564. 1927.

64. MAXIMOV, A., Über Chondriosomen in lebenden Pflanzenzellen. Anat. Anz. 43:241–249. 1913.

65. MAYER, A., RATHERY, FRANCIS, and SCHAEFFER, G., Sur les variations expérimentales du chondriome hépatique. Parallélisme entre la composition chimique du tissu et ses aspects cytologiques. Compt. Rend. Soc. Biol. Paris 76:398–402. 1914.

66. ――――, Les granulations ou mitochondries de la cellule hépatique. Jour. Physiol., Path. gén. 16:607–622. 1914.

67. McNAIR, J. B., Gum, tannin, and resin in relation to specificity environment and function. Amer. Jour. Bot. 17:187–196. 1930.

68. MILOVIDOV, P. F., Méthodes permettant la différenciation histologique des bactéries, des symbiotes et des chondriosomes. Bull. d'Hist. Appl. 5:382–391. 1928.

69. ――――, Observations vitales sur l'altération du chondriome chez le *Saprolegnia* sous l'influence de divers facteurs externes. Rev. Gén. Bot. 41:191–208. 1929.

70. ———, Zur Zytologie der Pflanzentumoren. Protoplasma 10:294–296. 1930.

71. MOTTIER, D. M., Chondriosomes and the primordia of chloroplasts and leucoplasts. Ann. Bot. 32:91–114. 1918.

72. NADSON, G. A., Über die Primärwirkung der Radiumstrahlen auf die lebendige Substanz. Biochem. Zeitschr. 155:381–386. 1924.

73. NADSON, G. A., and MEISL, N., Le mécanisme d'action du chloroform sur le protoplasme, le noyau, et le chondriome des cellules d'*Allium cepa*. Compt. Rend. Acad. Sci. Paris 183:150–152. 1926.

74. ———, le mécanisme d'action du chloroforme sur la matière vivante. Compt. Rend. Acad. Sci. Paris 183:82–83. 1926.

75. NADSON, G. A., and ROCHLINE-GLEICHGEWICHT, E. I., L'effet des rayons X sur le protoplasme et le noyau de la cellule végétale d'après les observations sur le vivant. Compt. Rend. Soc. Biol. Paris 94:249–251. 1926.

76. ———, Le chondriome est la partie de la cellule la plus sensible aux rayons X. Compt. Rend. Soc. Biol. Paris 95:378–380. 1926.

77. NEMEC, B., Über Pflanzentumoren. Archiv Exp. Zellforsch. 6:172–177. 1928.

78. ———, Tumoren an den Wurzeln der Pflaumen. Vestnik Kral Ceska Spol. Nauk. Prague 2. 1929.

79. NICHOLSON, F. M., An experimental study of mitochondrial changes in the thyroid gland. Jour. Exp. Med. 39:63–75. 1923.

80. NIXON, E. L., The migration of *Bacillus amylovorus* in apple tissue and its effect on the host cells. Pennsylvania Agr. Exp. Sta. Bull. 212. 1927.

81. PARAT, M., Contribution à l'étude morphologique et physiologique du cytoplasme. Archives d'anat. microsc. 24:73–357. 1928.

82. PINCKARD, J. A., Physiological studies of several pathogenic bacteria that induce cell stimulation in plants. Jour. Agr. Res. 50:933–952. 1935.

83. PINOY, P. E., À propos du cancer des plantes ou crown gall. Compt. Rend. Acad. Sci. Paris 180:311–313. 1925.

84. POLICARD, A., and MANGENOT, G., Action de la température sur le chondriome cellulaire. Compt. Rend. Acad. Sci. Paris 174:645–647. 1922.

85. RANDOLPH, L. F., Cytology of chlorophyll types of maize. BOT. GAZ. 73:337–375. 1922.

86. REGAUD, CL., Études sur la structure des tubes séminifères et sur la spermatogénèse chez les mammifères. Archives d'anat. microsc. 11:291–431. 1910.

87. RIKER, A. J., Some relations of the crowngall organism to its host tissue. Jour. Agr. Res. 25:119–132. 1923.

88. RIKER, A. J., BANFIELD, W. M., WRIGHT, W. H., KEITT, G. W., and SAGEN, H. E., Studies on infectious hairy-root of nursery apple trees. Jour. Agr. Res. 41:507–540. 1930.

89. ROBINSON, W., and WALKDEN, H., A critical study of crown gall. Ann. Bot. **37**:299–324. 1923.

90. ROSEN, H. R., Morphological notes together with some ultra filtration experiments on the crown-gall pathogene, *Bacterium tumefaciens*. Mycologia **18**:193–205. 1926.

91. RUDOLPH, K., Chondriosomen und Chromatophoren. Ber. Deutsch. Bot. Ges. **30**:605–629. 1912.

92. SAPEHIN, A. A., Untersuchungen über die Individualität der Plastide. Archiv Exp. Zellforsch. **13**:319–398. 1915.

93. SCHWARZ, W., Über die Ursachen und das Zustandekommen der Panaschierung bei einer Form der *Selaginella Martensii*. Protoplasma **10**:427–451. 1930.

94. SCOTT, W. J. M., Experimental mitochondrial changes in the pancreas in phosphorus poisoning. Amer. Jour. Anat. **20**:237–253. 1916.

95. SHEFFIELD, F. M. L., The formation of intracellular inclusions in solanaceous hosts infected with aucuba mozaic of tomato. Ann. App. Biol. **18**:471–493. 1931.

96. SHEFFIELD, F. M. L., and SMITH, J. H., Intracellular bodies in plant virus diseases. Nature **125**:200. 1930.

97. SMITH, E. F., BROWN, N. A., and McCULLOCH, L., The structure and development of crown gall, a plant cancer. U.S. Dept. Agr. Bur. Plant Ind. Bull. **255**. 1912.

98. SMITH, E. F., Bacterial diseases of plants. 1920.

99. ———, Le cancer des plantes ou crown gall. Rev. Bot. Appl. et d'Agric. Col. **5**:97–105. 1925.

100. SMITH, J. H., Virus diseases in plants. II. The amoeboid intracellular inclusions. Cambridge Phil. Soc. Biol. Reviews **5**:164–170. 1930.

101. TUNMANN-ROSENTHALER, Pflanzenmikrochemie. Berlin. 1931

102. TWISS, W. C., A study of plastids and mitochondria in *Pressia* and corn. Amer. Jour. Bot. **6**:217–234. 1919.

103. WRIGHT, W. H., and McCOY, E. F., An accessory to the Chambers apparatus for the isolation of single bacterial cells. Jour. Lab. Clin. Med. **12**:795–800. 1927.

104. WULFF, T., Studien über heteroplastische Gewebewucherungen am Himbeer- und am Stachelbeerstrauch. Ark. för Botanik **7**:no. 14. 1908.

105. ———, Weitere Studien über die Kalluskrankheit des Himbeerstrauches. Ark. Botanik **8**:no. 15. 1909.

106. ZIRKLE, C., The structure of the chloroplast in certain higher plants. Amer. Jour. Bot. **13**:321–341. 1926.

107. ———, The growth and development of plastids in *Lunularia vulgaris*, *Elodea canadensis*, and *Zea mays*. Amer. Jour. Bot. **14**:429–445. 1927.
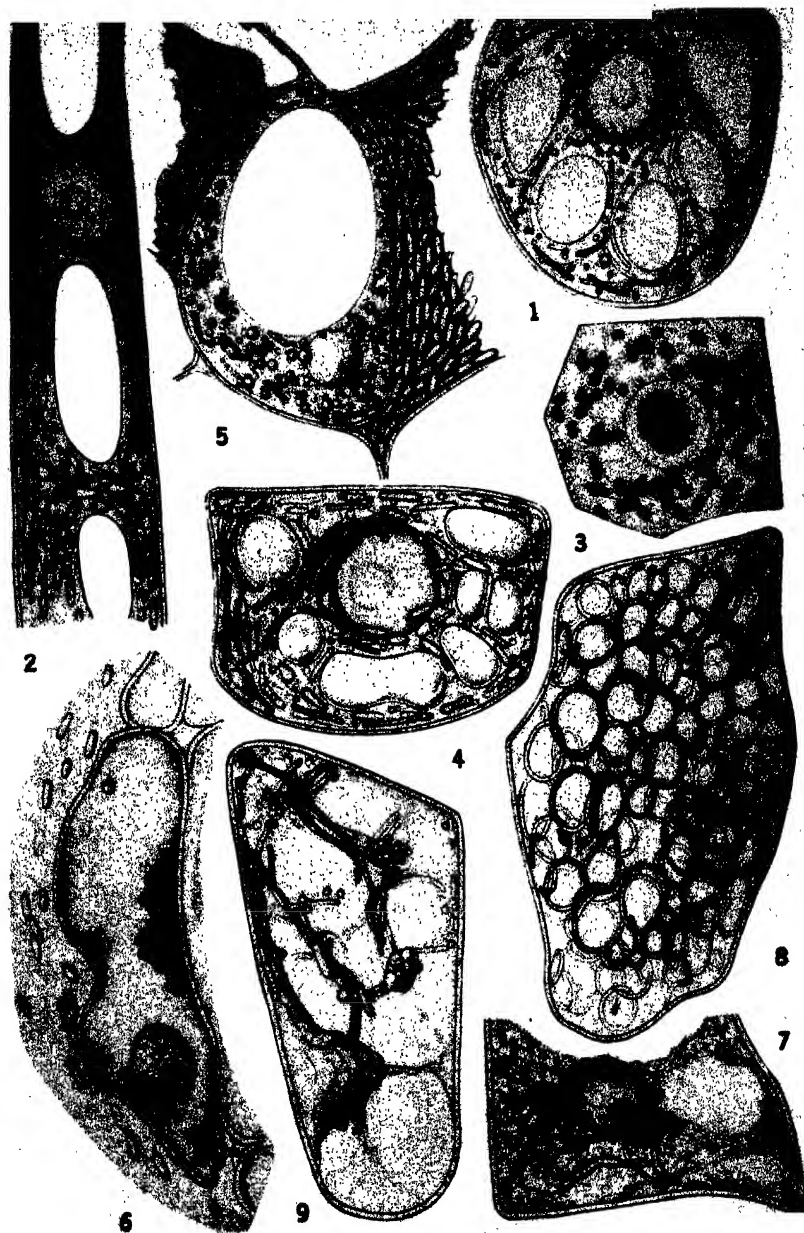
PLATE I

EXPLANATION OF PLATE I

All figures drawn at table level with Abbé camera lucida. Zeiss appochromatic 70×n.a. 1.25 water immersion objective with compensating 15 or 20× oculars used for figs. 1, 2, 4, 5, 6, 8, 9. Zeiss appochromatic 90× n.a. 1.3 oil immersion objective used for fig. 7; Spencer 100× fluorite objective used for fig. 3.

FIG. 1.—Typical cell from periblem of healthy root tip; the plastids are spherical, the mitochondria dispersed short threads. Fresh tissue section, unstained, 7.5% sucrose mount. ×1550 (approximately).

FIG. 2.—Typical cell from pleurome of healthy root tip as seen in unstained fresh section mounted in 5% sucrose solution; numerous plastids are of bacterial form and proportion. ×1550.

FIG. 3.—Cell from apical meristem of healthy root tip; plastids cannot be differentiated from mitochondria. NEMEC fixation, haematoxylin, balsam mount. ×2960.

FIG. 4.—Typical non-pathic gall tissue cell showing no visible difference between such cells and comparable cells of the root tip; elements of chondriome dispersed and very abundant; usually numerous plastids are of bacterial form and proportion; the small spherical fat globules are distinguishable by their higher refractivity. Fresh tissue section, unstained, 7.5% sucrose mount. ×2070.

FIG. 5.—Gall tissue cell recently encompassed by mass of bacteria; plastids have disappeared; mitochondria have diminished in number and the highly refractive fat globules have markedly increased in volume. Fresh section, unstained, 7.5% sucrose mount. ×2070.

FIG. 6.—Gall tissue cell which has been in contact with bacteria at edge of a bacterial cavity for a longer time; all elements of the chondriome have disappeared and fat globules are all that remain of the cytoplasm; the nucleus has a conspicuous granular appearance. Fresh section, unstained, 7.5% sucrose mount. ×2070.

FIG. 7.—Early stage of cellular disintegration from necrotic gall tissue. Some plastids have fused to form a reticulum of varying diameter. Erlicki fixation, haematoxylin, balsam mount, mitochondria not stained, not resolvable. ×2660.

FIG. 8.—Later stage of cellular disintegration from necrotic gall tissue. Dispersed elements of chondriome have fused to form a continuum or three dimensional netlike structure. Erlicki fixation, water mount. ×2070.

FIG. 9.—Plastids, mitochondria, and fat globules agglutinating into massive strand and solid condensation structures. Nucleus obscured by agglutinated mass of plastids over its surface. In autolyzing cells the identity of these elements frequently is lost in fusion structures. Fresh section unstained, 7.5% sucrose mount. ×2070.

DEVELOPMENTAL ANATOMY AND RELATIVE PERMEABILITY OF BARLEY SEED COATS

W. H. THARP

(WITH THIRTY-SEVEN FIGURES)

Introduction

Although much investigation has been centered upon determinations of the nature of the permeability phenomena of gramineous seed, most of it has been concerned only with the relative ability of various solutes to pass through the differentially permeable envelopes. Only two investigators have reported concerning the relative permeability of the grain itself. ORTON (17) has found varieties of *Zea mays* to differ in their relative permeability to several mercurial solutes in aqueous solution, and GUREWITSCH (13) has shown considerable variation in the degree of permeability among the individual kernels of an apparently uniform sample of one variety of wheat.

The investigations reported in this paper have been undertaken in an attempt to ascertain the extent of variation in the degree of permeability of the seed coat envelopes of common barley, special consideration being given the following questions: Do varieties of barley differ significantly in their relative degree of permeability? Does the environment during maturation influence the resultant permeability of the grain? Will prematurely harvested barley have an altered degree of relative permeability?

The seed of common barley, *Hordeum vulgare*, is known to be inclosed within a resistant, semipermeable envelope (3, 20, 7). However, a complete description of the origin, maturation, and ultimate condition of all the tissues contributing to the semipermeable envelope of the barley seed had not appeared in the literature at the time these investigations were begun.[1]

[1] The anatomical and physiological studies were started in 1929 at the University of Wisconsin and the investigations were continued while the writer held a National Research Council Fellowship at Cornell University, 1932–33. A preliminary report was presented before the physiology section of the Botanical Society of America at the annual meeting, Atlantic City, 1932 (22).

A thorough knowledge of the development of the caryopsis was deemed essential for the understanding of any effects that various changes of condition during growth might have upon its ultimate make-up.

I. Morphological and anatomical studies

A. DETAILS AT TIME OF FLOWERING

MATERIALS AND METHODS

During the growing seasons of 1929 and 1930, heads of Oder-brucker barley (Wisc. Ped. 5-1) growing at the Hill Farms of Madison, Wisconsin, were marked at the period of pollination. Kernels were then gathered daily and prepared for sectioning. In addition to Oderbrucker, kernels of three other hulled varieties and of four hull-less varieties were harvested at various stages of maturity. Transverse sections, 15 to 30 μ thick, of freshly harvested kernels were used for microchemical determinations. Heads and kernels to be imbedded in paraffin were killed and fixed in formal-acetic-alcohol in 1929 and in chrom-acetic medium in 1930. Materials were dehydrated by the ethyl alcohol-chloroform method in 1929 and by the butyl alcohol method in 1930. Silica was removed from the older heads and kernels by steeping for 36–60 hours in a solution of one part 50 per cent alcohol and one part commercial hydrofluoric acid. A combination of aqueous methylene blue, water-alcohol safranin, and orange G in clove oil was found satisfactory for the differential staining of sections of kernels gathered at the various stages of maturity. Sudan III in acidified 70 per cent alcohol and Sudan IV in lacto-phenol were employed to detect the presence of cutin and suberin. Microchemical analyses were made following the methods of ECKERSON (9) and HAAS and HILL (15). The accompanying illustrations, all prepared from microtome sections, consist of photomicrographs, drawings made with the aid of a camera lucida or a Promi projector, and enlargements of photomicrographs which were inked over and the residual photographic image then bleached away.

The single campylotropous ovule (24, 16) is attached near the distal end of the ovary and to the rachis side of the stylar canal by an elongate, reduced funiculus. The two integuments (fig. 1) are each two cell layers in thickness, except where the inner is bunched
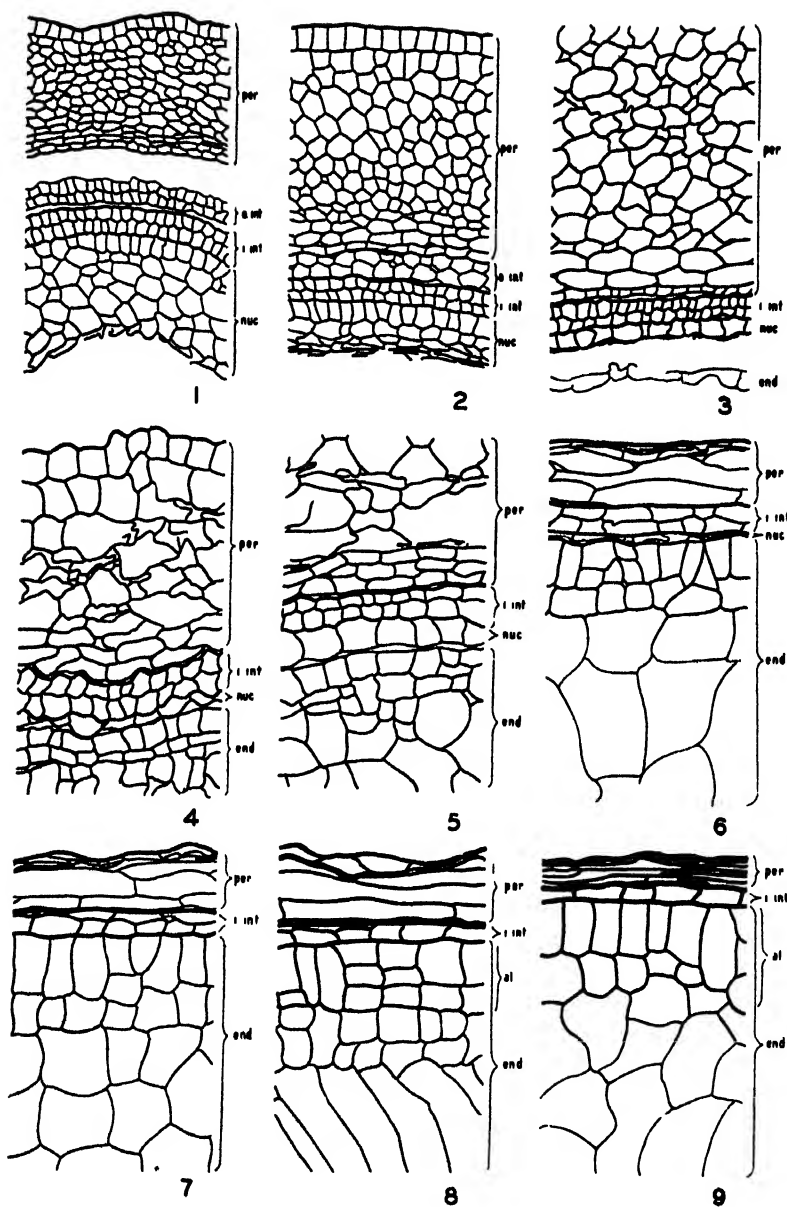
FIGS. 1–9.—Transverse sections of Wisc. Ped. 5–1 barley kernels showing seed coats and peripheral tissues at mid-lateral surface: fig. 1, just before pollination; fig. 2, 2 days after pollination; fig. 3, 4 days after pollination; fig. 4, 7 days; fig 5, 10 days; fig. 6, 13 days; fig. 7, 16 days; fig. 8, 20 days; fig. 9, mature kernel.

in the larger orifice of the outer integument at the micropyle (fig. 10). At this time there is no structural contact between the ovary, the two integuments, and the nucellar epidermis except at the chalaza. Sections of kernels at this age stained in acidified Sudan III show a thin red line of cutin on the inner and the outer epidermises of the ovary, a very faint trace of cutin on the inner and the outer epidermises of the outer integument, and a distinct cutin membrane on both the outer and the inner epidermises of the inner integument. The nucellar epidermis is without a definite cuticle at this stage. The main vascular supply to the ovary (usually seen as one large bundle but in many preparations distinctly of two separate and smaller bundles) passes up from the rachis, around the base of the ovary, and extends to the chalaza. The fruit is inclosed by the floral glumes and only partly fills the envelope formed by the imbricated lemma and palea.

B. DEVELOPMENT OF CARYOPSIS

ONE AND TWO DAYS FOLLOWING POLLINATION.—The developmental changes in the barley fruit are very rapid during the first two days following pollination. The kernel elongates rapidly and has attained its maximum length by the ninth day, while its maximum width is not attained until shortly before maturity. By the time the embryo consists of four cells, at the end of the second day (figs. 2, 14, 15), the micropyle has become closed and the entire nucellar epidermis is in contact with and adherent to the inner integument. The cutin membranes on the outer and the inner epidermises of the inner integument have thickened slightly and the cutin on the inner epidermis now seems to be common to both the integument and the nucellus. The growth of the inner integument has been accomplished mostly by cell division, little increase in cell size being noted. Although the outer integument has undergone much disintegration, the cells have been entirely absorbed only over the mid-abaxial surface. Increase in size of the pericarp has been mostly through enlargement of the cells, although in the region of the chalaza and the vascular bundle of the groove, cell division accounts for much of the growth and elongation. The inner epidermis of the pericarp is now well defined and the elongate cells adjacent to the epidermis mark the differentiation of the chlorophyll bearing tissue. The antipodal

cells and the free-nucleate endosperm form a thin layer in contact with the embryo and extend around the periphery of the enlarged nucellar cavity. The nucellus, except for the epidermis and one or two adjacent layers of cells, has been digested by the developing endosperm. In transverse sections of two-day-old kernels a group of glandular cells (the "sheaf cells" of COLLINS 7) can be discerned extending from the vascular bundle in the pericarp, through the chalazal region, and projecting fan-shaped into the nucellus.

THIRD TO SEVENTH DAYS.—By the fifth day of maturation the aleurone layer, composed of one to three rows of nearly rectangular cells, has been clearly differentiated. A primordial zone in the endosperm is now evident immediately interior to the aleurone. It is first active in the mid-posterior area but soon it initiates new cells from nearly the entire lateral periphery. By the seventh day of development there is a disorganized mass of endosperm and nucellar tissue in contact with the entire length of the chalaza. Another area of similar nature separates the endosperm and the still small (but slightly differentiated) embryo (fig. 18). These disorganized masses are composed partly of much hydrated cellulose and give a positive test for mucilage. The still persistent nucellar epidermis is in contact with the aleurone layer except where the latter is modified just interior to the chalaza and where it passes around the embryo as a single layer of cells. Cell division has now ceased in the outer row of cells of the inner integument, further enlargement of the integument being accomplished by the division of the cells of the inner row and elongation of the cells of both layers. The enlarged area at the micropyle has converged and the cutin membrane of the outer epidermis is now continuous over the micropyle. On the outer epidermis of the integument the cutin membrane has become somewhat thicker while the membrane on the inner epidermis has remained comparatively thin. In unstained transverse sections of five-day-old kernels (fig. 24), the walls of the cells in the chalazal region, and also some of the walls of the integument cells, are yellowish. This coloration is accompanied by change in wall composition, the cellulose becoming less easily hydrated and taking longer to dissolve in dilute, warm hydrochloric acid. The outer integument has entirely disintegrated and has been absorbed by the fifth day of development.

Within seven days the inner epidermis of the pericarp has also been mostly absorbed and the persistent portions crushed between the heavy walled chlorophyll tissue and the inner integument. There is now evidence of much crushing and breaking, with possibly some absorption, of the outer parenchyma of the pericarp (fig. 4).

LATER WEEKS OF KERNEL MATURATION.—Continued centripetal development of endosperm, accompanied by progressive enlargement and differentiation of the embryo, is instrumental in causing the enlargement of the caryopsis during the second and third weeks of maturation. By the thirteenth day (fig. 6) following pollination the kernel has attained nearly its maximum length but only about one-half of its mature transverse circumference. Subsequent development and enlargement of the caryopsis are due entirely to increase in the endosperm, the cells of which become gradually filled with starch. The embryo, still very small although well differentiated by the twelfth day, has attained nearly its full size by the end of the third week. The cells of the aleurone layer attain their full size by the sixteenth (fig. 7) day, and any further maturation is accomplished by formation of the protein grains and by thickening of the soft, cellulosic cell walls. Suppression of the nucellus is practically complete by sixteen days, and during later stages it is hardly distinguishable from the thick outer wall of the aleurone layer. By thirteen days (fig. 6) the inner integument has become differentiated into an outer row of thin walled, elongate cells and an inner row of thicker walled, less elongate cells. Even as early as eight days a brownish deposit is evident in the cells of the transchalazal strand and in the inner row of cells of the integument. Unstained transverse sections of thirteen-day-old kernels (fig. 25) show most of the cells filled with the deposit. The inner cutin membrane of the integument has remained comparatively thin while the outer membrane has markedly increased in thickness. At sixteen days (fig. 23) the outer membrane appears about twice the thickness of the inner cutin membrane. Growth of the endosperm causes compression of the pericarp and subsequent flattening of the outer row of thin walled cells, there being little or no content in these cells by the end of the second week of development. The cells of the chalazal region are nearly all filled with the fat deposits by the twelfth day and each cell

contains an inner suberin lamella, very thin but gradually thickening with maturity. The much elongated and very thick walled chlorophyll-containing cells are now the conspicuous portion of the pericarp.

MATURE KERNEL

INNER INTEGUMENT.—The mature caryopsis of barley (figs. 9, 20, 21) contains an embryo and endosperm completely inclosed by a resistant, cutinized, and semipermeable envelope (4, 20, 7). This membrane is composed of the inner integument, both inner and outer epidermises of which are cuticularized, and a suberized strand of chalazal cells connecting the flanks of the integument. The persistent inner integument is composed of two layers of cells. The outer epidermis of the inner integument, composed of a single layer of thin walled elongate cells which are nearly devoid of contents, has become crushed against the single layer of thick walled cells of the inner epidermis (fig. 9). In transverse section most of this integument thus appears to be composed of a single layer of cells with greatly thickened outer peripheral walls, since the lumena of the cells of the outer epidermis are discernible only in a few places where there has been less crushing. Most of the thicker walled cells of the inner row contain the remnants of their protoplasts, but for the most part the cells are filled with the fat deposits mentioned earlier. These deposits are found around the entire periphery of most kernels but in others they are absent from the cells over the anterior surface of the endosperm and sometimes over the embryo. Hull-less varieties, considered as a group, were found to have less of this substance deposited in the cells of the inner integument (figs. 27–29). It is also significant that the deposits were absent from the cells over the embryo, around the micropyle, and the abaxial surface of the endosperm more often in the hull-less than in the hulled varieties. The inner integument of the mature kernel is invested with a thin cutin membrane on its inner epidermis and a much thicker membrane on the outer surface (figs. 27–32). This membrane and the entire integument are not uniform in thickness. It is much the thickest where it folds over the apex of the kernel. It is very thick in the flanks of the furrow near the chalaza and is again thick near the grouped cells at the micropyle. It is slightly thinner over the lower anterior surface

than over the upper anterior and lateral surfaces, but it is distinctly thinner over the embryo. It is much the thinnest in the small area immediately opposite the micropyle (fig. 11).
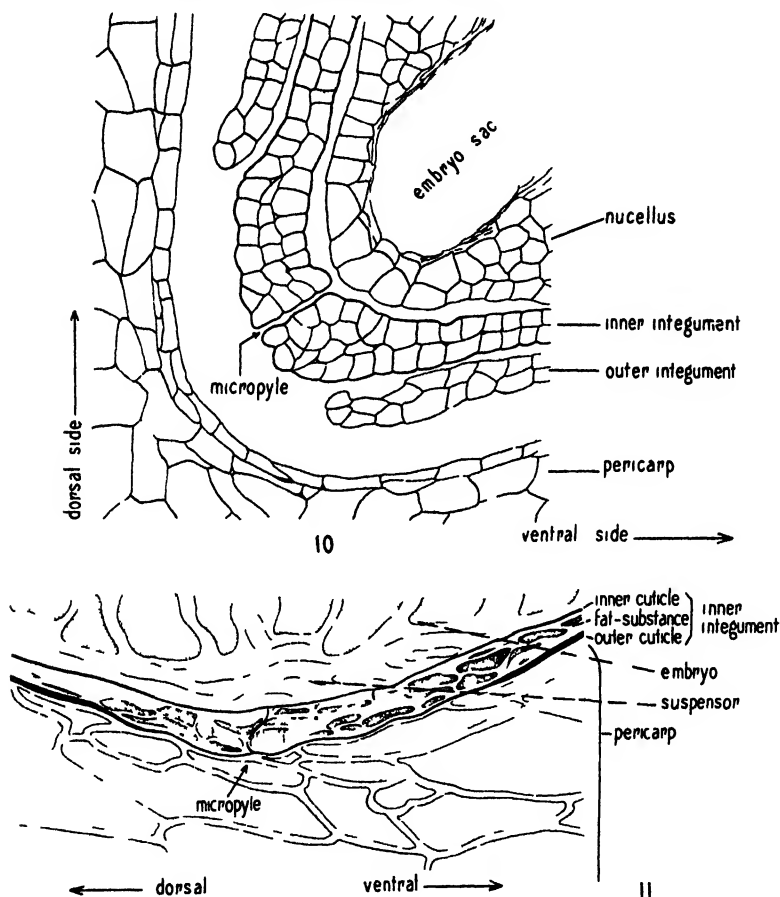
FIGS 10, 11 —Fig 10, median, radial, longitudinal section through micropyle of barley kernel (Wisc Ped 5-1) just before pollination. Fig 11, same of mature kernel of White Hull-less barley

CHALAZA.—The cells of the chalaza are filled with fat deposits,[2] the walls are yellowish, and there is an inner lamella of suberin in each of these closely packed, thick walled cells.

[2] When the fat deposits are first formed they can be dissolved away with warm, alcoholic potassium hydroxide As they increase in density and begin to fill the cells during

EMBRYO AND ENDOSPERM.—The embryo is oriented with its coleorhiza at the micropyle, pointing almost directly down parallel to the rachis, and makes contact with the micropyle by means of its group of empty suspensor cells. The scutellum of the embryo slants obliquely across the base of the kernel with its apex against the periphery at a point about one-third distant from the proximal end (fig. 20). The inner integument follows closely the curvature of that portion of the embryo which it directly covers. There is still a wide area of disorganized tissue between the scutellum and the starchy endosperm, clearly a result of digestion by the growing embryo, and a narrower line of similar nature extending the length of the chalaza and separating the "sheaf cells" from the aleurone layer.

ALEURONE.—The aleurone cells are thick walled and rectangular in outline, containing closely packed protein grains and rather large nuclei. The aleurone layer just interior to the "sheaf cells" is modified and its cells are somewhat disorganized. It passes around the embryo as a single layer of elongate cells, except at the micropyle where it ends at the margin of the compressed suspensor cells of the embryo.

NUCELLUS.—Remnants of the nucellus are discernible near the chalaza and over the top of the endosperm. Elsewhere this tissue has been absorbed or crushed until its remains appear to be part of the thickened peripheral walls of the outer row of aleurone cells. This layer of cells is now sealed to the cuticle of the inner epidermis of the inner integument.

PERICARP.—The thick walled cells of the pericarp are now sealed to the cuticle on the outer epidermis of the inner integument. The rest of the pericarp has been reduced, by absorption and crushing, to a few rows of collapsed parenchymatous cells and a much flattened outer epidermis with its cuticle. The vascular supply to the fruit

the later weeks of maturation, they become gradually more resistant to fat solvents. When the kernels are mature a prolonged heating in alcoholic potassium hydroxide will cause solution of much of this substance, but in the cells within and adjacent to the chalaza, and also in the cells around the micropyle, there often remain resistant outer lamellae of the original deposits. At no time during kernel development will this substance stain red in acidified Sudan III or Sudan IV, except for the very outer shell which may take the stain at its periphery. The exact composition of this substance has not been investigated during these studies.

extends the length of the groove, being surrounded on its posterior and lateral sides by crushed pericarp parenchyma and in contact on its anterior side with the glandular cells just exterior to the chalaza. The cuticle of the outer epidermis is adherent to the inner cuticles of the floral glumes where the latter are in contact with the caryopsis.
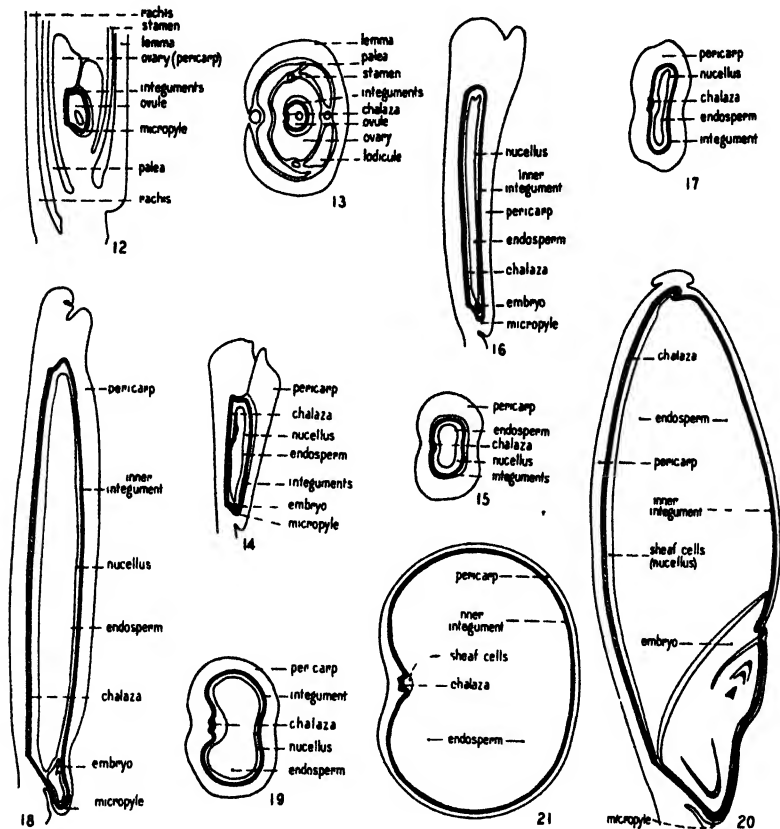

FIGS. 12–21.—Diagrams of longitudinal and transverse sections of Wisc. Ped. 5–1 barley kernels at five stages of development (palea and lemma have been omitted, except in figs. 12 and 13, of flowers fixed at time of pollination): figs. 14, 15, 2 days after pollination; figs. 16, 17, 4 days; figs. 18, 19, 7 days; figs. 20, 21, mature kernel.

PROBABLE RELATION OF SEMIPERMEABLE ENVELOPE TO VARIATIONS IN DEGREE OF PERMEABILITY

The results of these investigations are in agreement with those of COLLINS (7) and KRAUSS (16), in that the semipermeable envelope

of the mature caryopsis was found to consist of the inner integument, with outer epidermis heavily cuticularized and the inner epidermis lightly cuticularized, and the resistant suberized cells across the length of the chalaza. It is also agreed that the thickness of the outer cuticle of the integument is not uniform over the entire seed, it being relatively thin in the region over the embryo. These previous investigators have found also that the two membranes were originally developed by the integument before the time of flowering. COLLINS has suggested that the tract of chalazal tissue where it meets the dorsal rim of the scutellum might be a possible point of easy permeability. The present investigations and those of KRAUSS have discovered no structural weakness at this point indicative of easy permeability, but rather that the thin membrane across the micropyle (COLLINS' second point of probable easy permeation), and to some extent the relatively thin covering over the embryo, are structurally the most logical points of probable easy permeation. KRAUSS has pointed out that in naked barleys the cells of the integument at the micropyle do not usually contain the fat deposits found elsewhere in this tissue. He has also suggested that in some varieties the outer cuticle and at times the inner cuticle may be entirely absent from the micropylar area. In the four hull-less varieties (C. I. nos. 4360, 4344-2, 4339-1, and White Hull-less) used in these investigations the micropylar area is usually crossed by at least a very thin membrane on the outside and on the inside of the integument at this point. It is true that the cutin deposit of the integument is relatively more apparent in immature kernels but seldom is it absent from this area of the mature kernels investigated. In some hull-less varieties there are found more kernels with but little of the fat substance in this group of integument cells, but in any variety studied the deposits are more often absent from the cells over the embryo and the anterior surface than from the region across the micropyle. KRAUSS has reported that in many kernels of naked barley the cells of the integument at the micropyle could be dissolved away with strong acids which have little effect on the cutin membranes which he believed to be absent in these cases. The writer steeped 20 mature kernels of each of the four hull-less varieties investigated in 60 per cent hydrochloric acid for 24 hours at room temperature. From one
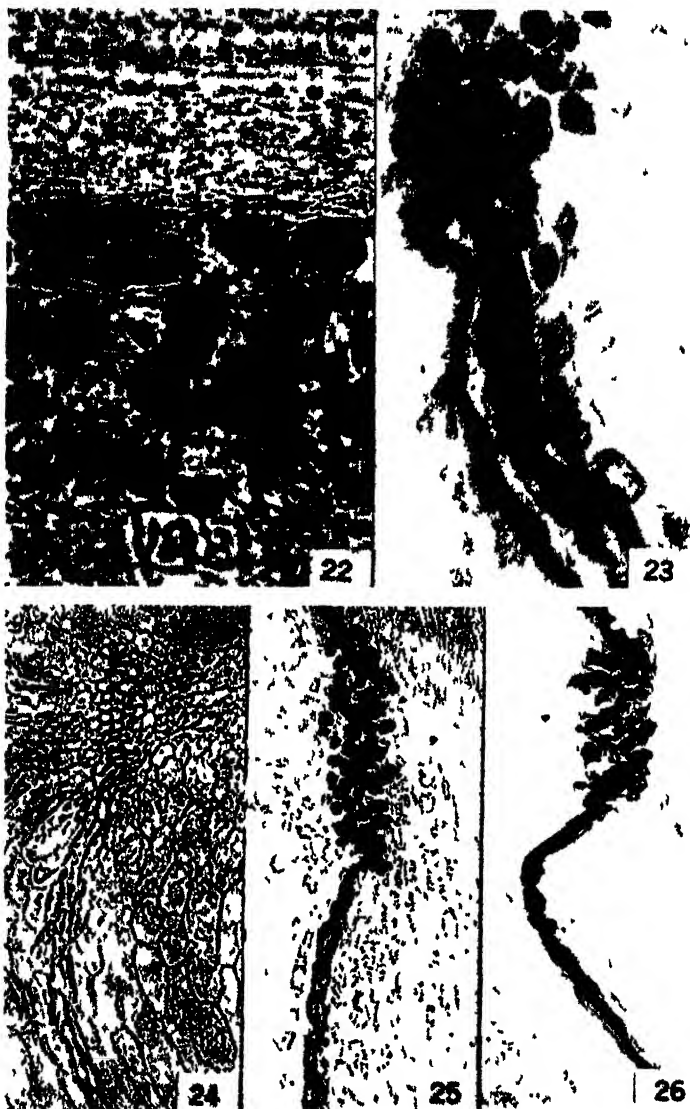
FIGS 22-26 —Fig 22, longitudinal section through chalazal region of mature kernel, stained in acidified Sudan III Note distinct suberin lamella of chalazal cells and large deposits of fatty substance Vascular bundle of pericarp seen at top of photograph ×1250. Fig 23, transverse section of kernel fixed 16 days following pollination, stained in acidified Sudan III The two cutin membranes of integument extend well into chalaza. Pericarp is toward left of photograph ×580 Figs 24-26, unstained, transverse sections of barley kernels showing natural staining of cell walls and fat deposits within cells of inner integument and chalaza fig 24, fixed at 5 days, fig 25, at 13 days; fig. 26, at maturity Pericarp toward right of photographs ×270.

to three of the kernels of each variety had the embryo entirely dissolved by the end of this time. The remaining 17-19 kernels of each lot were swollen and distended over the embryo end, and subsequent examination of the embryo showed it to be unaltered by any solvent action of the acid. It must then be concluded that about 90 per cent of the kernels of any variety investigated[3] contain sufficient cutin deposited over the micropyle to inhibit the entrance of 60 per cent hydrochloric acid for 24 hours and thus demonstrate the possession of semipermeability dependent here upon the cutin membranes.

In each of the eight varieties of barley investigated, it has been found that from the time of flowering to maturity there was a gradual and progressive thickening of the outer cutin membrane of the inner integument. It might then be expected that prematurely harvested kernels would contain a thinner deposit of cutin on the integument than fully ripened kernels, and subsequently be more readily permeable. It was found also that there was a relative difference in the thickness of these membranes among different varieties of barley, again suggesting a variation in degree of relative permeability. Another variable which might be responsible for resultant differences in permeation rates is the amount of the fat deposits within the cells of different areas of the integument. While the comparative differences of varieties relative to each of these factors suggest a possible correlation of permeation with structure, it might again be possible that there exist within the cutin membranes some differences in chemical and physical composition which are the controlling factors concerning the subsequent relative permeability of the seed envelopes.

II. Permeability studies

A. RELATIVE RATES OF ABSORPTION FROM AQUEOUS SOLUTIONS BY BARLEY KERNELS

MATERIALS AND METHODS

The same eight varieties of barley used for the morphological studies were employed in the permeability investigations. In the summer of 1930, and again in 1931, one lot of each variety was grown under prevailing cultural conditions at the Hill Farms, Madison,

[3] These varieties of hull-less barley are not the same as those used by KRAUSS (16).

Wisconsin. Another lot of each variety was grown under a field moist-cage where high humidity, high soil moisture content, and low light intensity were maintained throughout the period from flowering to complete maturity. The samples grown under field conditions are here termed as from a "dry" environment and those matured under the moist-cage as from a "wet" environment, these terms being only relative. Four harvestings were made from each of the varieties grown in the two environments as follows: at 14, 21, and 28 days following flowering, and at maturity. A portion of each prematurely harvested sample was preserved in 70 per cent ethyl alcohol and the remainder of the samples were allowed to air-dry in the laboratory.

The increase in weight of kernels steeped in various solutions has been used by Brown (3, 4) and many other investigators as a measurement of the rate of permeation of solvent and solute. This method has been adopted, with modifications, to determine the differences in permeability among different varieties of barley and also those differences induced by the environment during maturation. Three different steeping solutions have been employed: (1) distilled water which permeates rapidly, (2) an aqueous solution of iodine-potassium iodide which permeates but fairly rapidly, and (3) various concentrations of sodium chloride which permeate with exceeding difficulty. Duplicate 100-kernel samples of each lot were steeped at 25° C. in 250 cc. Pyrex beakers containing 150 cc. of the solution. Those samples steeped in water and in iodine-potassium iodide were removed at regular intervals of 15, 30, 45, and 60 minutes and then at the end of 2, 3, 6, 9, 12, 24, and 32 hours. The samples steeped in distilled water were drained and then dried by rolling between towels for one minute. They were then centrifuged at high speed for one minute in wire baskets over absorbent cotton (fig. 33) and weighed within the first minute following removal from the centrifuge. Since it is absorption by the seed, exclusive of that absorbed by the pericarp, that it is desired to calibrate, this method aids in rapidly freeing the pericarp of moisture while the semipermeable membranes inhibit any appreciable loss from the seed. The samples steeped in a solution of 1 per cent iodine in 10 per cent potassium iodide were drained and immediately washed in one change of water. These

washed kernels were placed in a 10 per cent solution of sodium thio-sulphate for three minutes, the time necessary to clear most of the iodine from the tissues of the pericarp. The cleared kernels were washed in four changes of tap water and then dried and weighed

HULL-LESS BARLEY   HULLED BARLEY
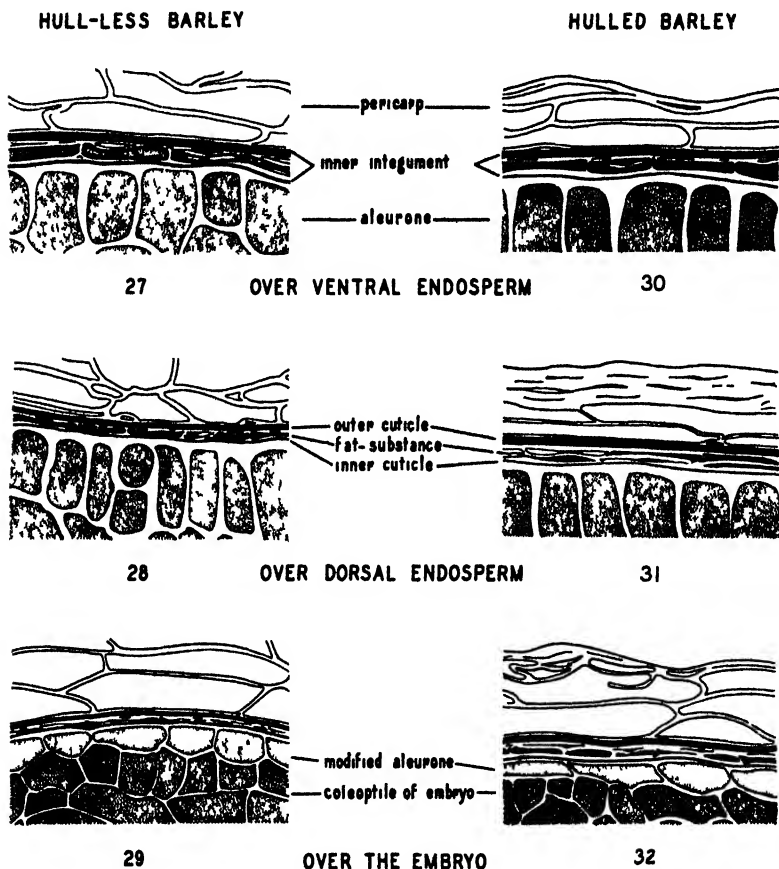
FIGS. 27–32.—Transverse sections of two varieties of barley showing structure of mature seed coat membranes over ventral (posterior) and dorsal (anterior) endosperm, and over embryo: figs. 27–29, White Hull-less barley; figs 30–32, Wisc. Ped. 5–1 barley.

with the same procedure. The following methods were employed to determine the effect of concentration of sodium chloride upon the relative absorption by different lots of barley. Duplicate 100-kernel samples of each lot were placed in each of nine differing concentra-

tions of sodium chloride and all samples were steeped for 24 hours at
25° C. At the end of this time the samples were drained, washed in
running water for three minutes to free the salt from the tissues of
the pericarp, then dried and weighed following the same technique
used for the samples steeped in water. It was found that the kernels
taken from the concentrated salt solutions would increase in weight
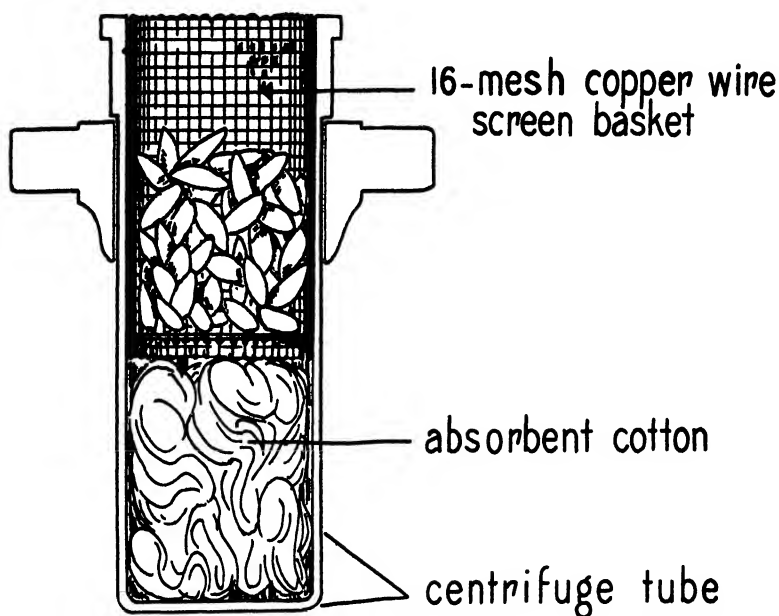during the three minute washing. To eliminate the discrepancy in-

FIG. 33.—Centrifuge tube-holder adapted for rapid removal of adhered moisture
from steeped barley kernels.

troduced by this procedure, the average amount which air-dry seed,
of the same lot, would absorb during this after-treatment was sub-
tracted from the total amount absorbed. This correction was applied
to the result from each sample, even the ones steeped in zero sodium
chloride (water). Table I gives the data obtained in one of these
experiments using samples of Spartan barley, from both the wet and
the dry environments, steeped in distilled water. The results of all
the steeping experiments are represented graphically by figure 34.

It is obvious, considering the results shown in table I and figure 34,

that a relatively wet environment during maturation induces a greater degree of permeability of the seed coats and a greater absorptive capacity of the seed. At any one time after the first 15 minutes of steeping there has been more water absorbed by the wet than by the dry sample of any of the varieties tested. Another interesting relation is shown when the grain is allowed to absorb water until a constant weight has been attained. When the samples have thus

TABLE I

ABSORPTION OF WATER BY TWO DIFFERENTLY MATURED LOTS
OF SPARTAN BARLEY (25° C)

| TIME OF STEEPING | DRY MATURATION | | | WET MATURATION | | |
| | WEIGHT OF 100 KERNELS (GM) | | | WEIGHT OF 100 KERNELS (GM) | | |
	BEFORE STEEPING	AFTER STEEPING	PERCENTAGE GAIN	BEFORE STEEPING	AFTER STEEPING	PERCENTAGE GAIN
15 sec.	4 9931	5 3483	7 11	4 4109	4 8142	9 14
30 "	5 0000	5 4626	9 25	4 4390	4 9581	11 69
45 "	5 1026	5 6957	11 62	4 4413	5 0221	13 07
60 "	4 9757	5 6087	12 72	4 2810	4 9364	15 30
2 min	4 8244	5 6235	16 56	4 4248	5 2628	18 93
3 "	5 0037	5 8975	17 86	4 4863	5 4539	21 56
6 "	5 0007	6 1932	23 84	4 3766	5 7271	30 85
9 "	5 1516	6 5964	28 04	4 4370	5 9560	34 23
24 "	5 0158	7 2798	45 13	4 2370	6 5673	53 69
33 "	5 0521	7 6864	52 14	4 4122	7 0600	60 01

reached an equilibrium with the solution, the wet samples of each variety have absorbed a greater amount of water than the comparable dry samples, even though the average weight of the wet samples is less than that of the dry samples. A comparison of the curves of absorption by Wisc. Ped. 38 barley shows that in a given variety the more rapid rate of permeability and the greater absorptive capacity of the wet samples remain relatively constant, whether the kernels have been steeped in pure water (permeating easily), in iodine-potassium iodide (permeating less easily), or in sodium chloride (scarcely permeating). Each variety tested showed a different relative degree of permeability proportionally maintained in the three types of solutions. The differently matured samples of the varieties
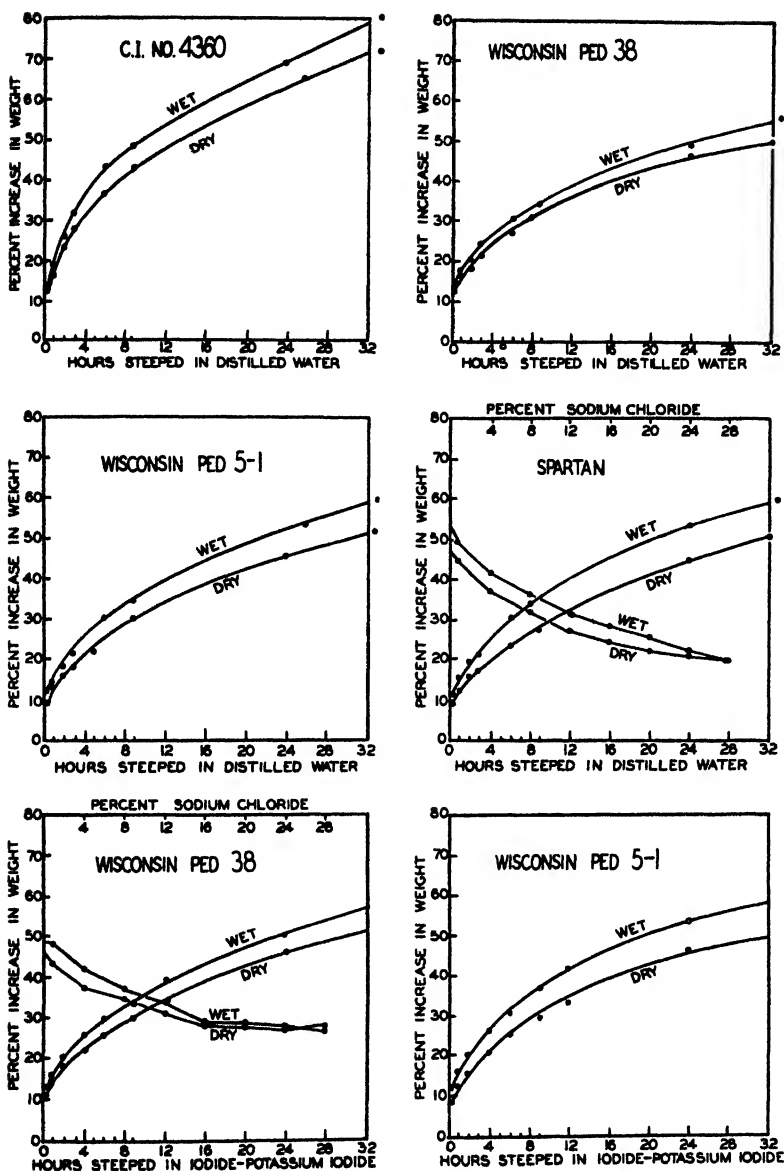
FIG. 34.---Relative rates of absorption from aqueous solutions by barley kernels (temperature 25° ±1° C.). Each point on curves represents average absorption by two 100-kernel samples. Samples of wet kernels were matured under field moist-cage; dry samples were matured under prevailing cultural conditions.

steeped in the sodium chloride solutions exhibited divergent degrees of absorption in the dilute solutions but failed to differ materially in this respect when steeped in the more concentrated solutions.

B. PERMEABILITY OF BARLEY SEED COAT MEMBRANES

GOLA (11, 12), SHULL (21), DENNY (8), ORTON (17), and others have employed seed coat membranes as septa of variously constructed osmometers. This procedure furnishes an accurate method of determining the permeability of the seed coat without involving other complex forces operative during absorption. These methods of diffusion are rather slow, and because considerable variation is encountered in any one carefully selected lot of grain, it was desired to find a method whereby large numbers of membranes could be tested for any one determination.

OSTERHOUT (18) has measured the permeability of living cells by placing together many discs of tissue and then testing their resistance to electrical conductivity. STILES and JØRGENSEN (22) have criticized this method on the assumption that an electric current would induce marked changes in the protoplasmic membranes of the living cells. This objection is of course not applicable to the non-living semipermeable membranes of the barley kernels. GUREWITSCH (13) has adopted the OSTERHOUT principle using seed coat membranes of wheat as septa in an osmometer with the electrodes completely separated by the membrane. He tested one variety of wheat kernels in many solvents and his results seem significantly quantitative when great numbers of membranes from any one sample were tested for one average determination. This last method has been adopted, in somewhat modified form, for these investigations.

C. RESISTANCE OF SEED COAT MEMBRANES TO CONDUCTIVITY IN SOLUTIONS OF ELECTROLYTES

MATERIALS AND METHODS

About 150 membranes were prepared from each of the variously harvested samples of the eight varieties of barley used in these experiments. Membranes were also prepared from the kernels, which were killed and preserved in alcohol immediately after premature

harvesting. The carefully selected kernels were soaked in water at 32° C. for twelve hours, after which time the hulls could be removed and the endosperm was sufficiently soft to be cut easily. The tip and base of each kernel were cut off transversely and the hardened tissue of the groove removed by a longitudinal cut along each flank. These cut kernels were placed in a 10 per cent solution of taka-diastase and allowed to digest at 38° C. for 60 hours. The softened starchy endosperm was then easily removed and the membranes were thoroughly washed in running water. The finished membranes were stored in 30 per cent ethyl alcohol until tested. Each prepared membrane consists of the following tissues: (1) the intact aleurone with fragments of the endosperm cells; (2) the remnants of the nucellus; (3) the inner
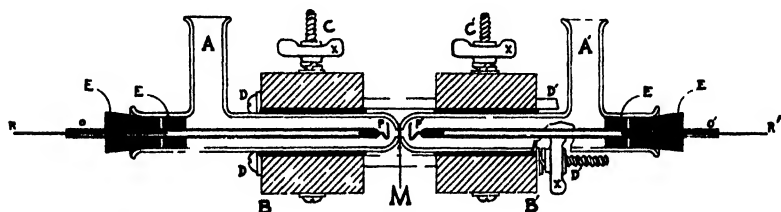

FIG. 35.—Special cell for testing resistance of barley seed coat membranes to electrical conductivity in solutions of electrolytes: M, test membrane placed between ground edges of two tubes (A,A') and tightened in place by four set screws (D,D'). The tubes of Pyrex glass are filled with test solution through openings at A and A'. The two platinum electrodes (ROP, $R'O'P'$) are held in place by sealed-in stoppers (E). Tubes held firmly in the blocks by hinges on one end and by set screws (C,C') on the other end. Tubes may be separated for insertion and withdrawal of membranes by loosening set screws (D,D'). Current supplied at electrode leads (R,R'). Conductivity surface of electrodes (P,P') is about 18 sq. mm. Electrodes operate at a distance of 23 mm.

integument with its thin inner and thick outer cutin membranes; and (4) the pericarp with the epidermis often torn away during preparation. The aleurone and pericarp were left on the finished membranes because their added thickness facilitated the sealing of the membranes between the two ground edges of the osmometer tubes. Actual tests have demonstrated that the removal of either or both of these layers causes only a negligible change in their resistance to conductivity. The apparatus designed and used for testing the resistance of these seed coat membranes to conductivity is shown and described in figure 35.

TESTING RESISTANCE

Each lot of membranes to be tested was removed from the alcohol, thoroughly washed, and then placed in distilled water. In conducting an individual test a membrane was first steeped in the test solution for three minutes in order to obtain complete infiltration. After this pre-soaking the membrane was securely clamped between the ground edges of the tubes, the tubes wiped dry at their junction, and each tube filled with solution so as to cover the electrodes completely. The wheatstone bridge was then connected to the electrodes and the resistance determined by the Kohlrausch method (10). While one reading was being taken the next membrane was placed to soak, and so on. Special care was exercised when inserting a membrane so as to avoid leakage during the test, because even a minute leak at this juncture would result in a capillary film of liquid around the membrane edge, thus lowering the resistance reading to a noticeable degree. Considerable variation within any one lot of barley membranes, or even at different locations on the same membrane, necessitated twenty or more separate determinations for every lot of membranes tested. These initial readings were later checked with additional readings using a second group of membranes prepared from the same lot of barley. If any discrepancies were noted between these two sets of data, additional readings were taken until an accurate average could be established. Trichloracetic acid has been chosen as a test solution because it is known to be an electrolyte which permeates easily (4). Other electrolytes have been used to establish the fact that the results are not peculiar to the one solution.

The data in table II and the resistance graphs in figure 36 indicate that for each variety of barley tested there is a definite, average degree of permeability; that Spartan is the least permeable and C. I. no. 4360 the most permeable, with the other varieties exhibiting various degrees between the two extremes; that the effect of increased moisture, high humidity, and low light intensity during maturation is a greater degree of permeability in all the varieties except C. I. no. 4339–1.[4] The results obtained with three of the

4 Similar tests of the differentially matured samples of this variety, using materials grown the preceding year (1930), gave a difference in the same relative proportion

varieties, grown under the two differing environments and harvested at the various periods during growth, are presented graphically in figure 37. The data are not complete for the entire series, nor for any one variety, because in many cases the membranes stored in alcohol were dried down and could not be tested comparably with the rest of the stored samples.

TABLE II

RELATIVE RESISTANCE OF EIGHT VARIETIES OF BARLEY, MATURED UNDER TWO
DIFFERENT ENVIRONMENTS, TO ELECTRICAL CONDUCTIVITY IN
NORMAL TRICHLORACETIC ACID ($25° \pm 1°$ C.)

SAMPLE	NUMBER OF MEMBRANES TESTED	READINGS IN OHMS RESISTANCE		
		HIGHEST	LOWEST	AVERAGE
Spartan				
Dry....................	20	2300	1200	1782
Wet....................	25	1900	930	1373
Wisc. Ped. 5-1				
Dry..........	32	1980	1100	1663
Wet.......	27	2000	1000	1399
Trebi				
Dry....................	33	1900	1000	1401
Wet....................	30	1900	900	1246
Wisc. Ped. 38				
Dry	36	2000	900	1341
Wet....................	41	1900	800	1199
C.I. no. 4344-2				
Dry....................	31	1700	1100	1329
Wet....................	33	1400	1000	1172
C.I. no. 4339-1				
Dry....................	33	900	620	762
Wet....................	36	990	450	764
W. H.				
Dry....................	30	800	510	660
Wet....................	24	720	520	631
C.I. no. 4360				
Dry....................	30	580	300	408
Wet....................	32	420	280	336

These data show that there is an increase in resistance of the seed coats correlated with the increased periods of maturation. That there is an early effect of the environment upon the subsequent permeability is shown by the differences in resistance of the samples of

shown by other varieties grown in 1930 and in 1931. The nearly exact average resistance of the membranes of the two lots may be due to the fact that they were inadvertently prepared from the same lot of barley.
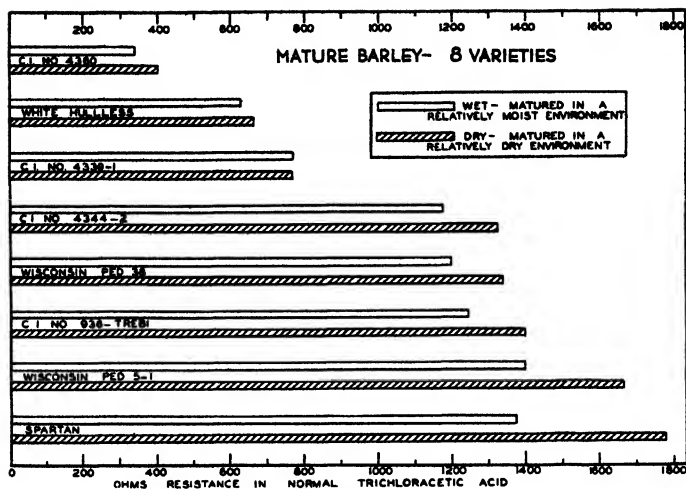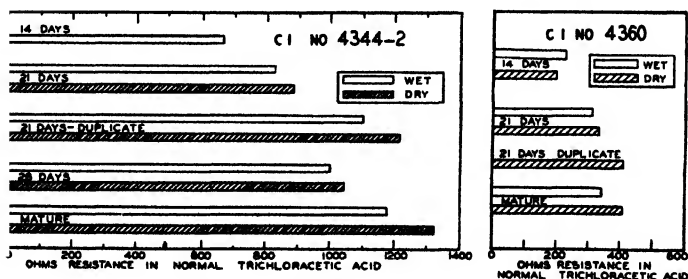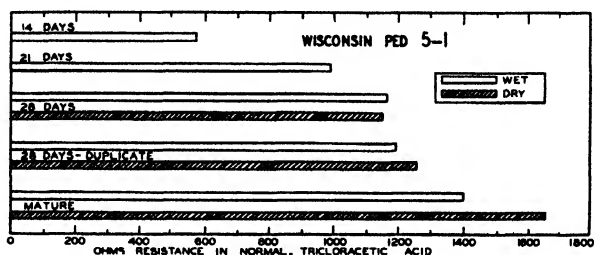
FIG 36

FIG. 37

FIGS. 36, 37.—Fig. 36 (above), relative resistance to conductivity of differently matured samples of eight varieties of barley. Resistance tested in normal trichloracetic acid at 25° ± 1° C. Each column represents average resistance of 25 or more individual membranes. Fig. 37 (below), relative resistance to conductivity of differently matured samples of three barley varieties, harvested at 14, 21, 28 days following pollination, and at maturity. All samples preserved in 70% alcohol when gathered, except 'Duplicate' samples which were first allowed to become fully air-dried. All samples tested in normal trichloracetic acid at 25° ± 1° C.

C.I. nos. 4360 and 4344–2 which were harvested at 21 days following flowering. Another striking result is evident in the relative resistance of the samples which were prematurely harvested and then allowed to dry (duplicate). The resultant resistance to permeation is higher than that of the comparable samples which were preserved in alcohol when harvested and is nearly as high as that of the fully matured samples. The results of an early harvest are not the same for each of the varieties tested although the general trend is similar. It should be noted that the samples of Wisc. Ped. 5–1 and of C.I. no. 4344–2 have a similar relative resistance at fourteen days, and then at maturity their permeability values are divergent. The samples of C.I. no. 4360 at any chosen stage of development were found to be much more permeable than the comparable samples of the other two varieties. It is evident that there is a wide divergence in permeability among different barley varieties at maturity, and that the rate at which resistance of the seed coats is increased during maturation is not the same for all varieties. This might be due, in some measure, to the fact that the high resistance of the seed coat membranes of some varieties is a possible development of the later part of the maturation period.

RESISTANCE OF BARLEY SEED COAT MEMBRANES TO CONDUCTIVITY IN SOLUTIONS OF INORGANIC ELECTROLYTES

The relative differences in seed coat permeability, which were obtained with trichloracetic acid as the test solution, were encountered when tested in solutions of inorganic electrolytes. The data in table III give a comparison of the results obtained with two lots of membranes tested in sodium chloride and in trichloracetic acid. A comparison of the relative resistance of one lot of membranes to conductivity in trichloracetic acid and in various solutions of inorganic electrolytes is shown by the data presented in table IV.

The data in table IV show that significant differences in relative permeability of seed coat membranes can be demonstrated by the use of solutions of solutes which permeate with difficulty. The differences are not exactly proportional in relation to those obtained using trichloracetic acid, it being obvious that non-permeating solutions such as sodium chloride do not offer an accurate means of determin-

ing slight differences by the conductivity method of testing. The inorganic acids tested permeate more slowly than trichloracetic acid, even though the concentrations were adjusted to give the same

TABLE III

RELATIVE RESISTANCE TO CONDUCTIVITY OF TWO SAMPLES OF BARLEY
SEED COAT MEMBRANES TESTED IN TRICHLORACETIC ACID
AND IN SODIUM CHLORIDE (25° C.)

SAMPLE OF MEMBRANES	TESTED IN 2.N SODIUM CHLORIDE			TESTED IN NORMAL TRICHLORACETIC ACID		
	No. OF READINGS	READINGS IN OHMS RESISTANCE		No. OF READINGS	READINGS IN OHMS RESISTANCE	
		WITHOUT MEMBRANE	AVER. OF MEMBRANES		WITHOUT MEMBRANE	AVER. OF MEMBRANES
C. I. no. 4360 wet........	20	37	6,770	20	29.2	389
Trebi dry....	20	37	9,850	20	29.2	1330

TABLE IV

RELATIVE RESISTANCE TO CONDUCTIVITY OF SEED COAT MEMBRANES
FROM WISC. PED. 5–1 BARLEY IN SOLUTIONS OF INORGANIC
ACIDS, BASES, AND SALTS (25° C.)

SOLUTION	CONCENTRA-TION	No. OF READINGS	READINGS IN OHMS RESISTANCE	
			WITHOUT MEMBRANE	AVER. OF MEMBRANES
Trichloracetic acid.........	1.n	24	31.2	1,368
Nitric acid	0.275 n	24	31.2	2,770
Hydrochloric acid	0.25 n	24	31.2	3,600
Potassium hydroxide.......	0.5 n	12	31.2	3,150
KOH (membranes steeped in solution 5 min.).........	0.5 n	12	31 2	776
Potassium iodide..........	10%	24	78	9,840
KI plus 1% I₂......	10%–1%	24	147	1,890

initial conductivity reading of 31.2 ohms. Potassium hydroxide, also with the same initial reading, encounters a similar high resistance in the membranes until it has apparently had some solvent action upon the cutin membranes (evidenced by the lower resistance after pre-

soaking). Since iodine-potassium iodide has been found to permeate readily in absorption experiments, it is rather to be expected that it is found to permeate more rapidly in these tests than the potassium iodide having a much lower initial conductivity (table IV). Just why the addition of the metallic iodine should increase the permeability to the resultant solution is not fully understood, but it seems possible that it may be due partially to the complex nature of the double salt thus formed.

The data in table IV show a probable solvent action of potassium hydroxide when the membranes were immersed in the solution for a

TABLE V

EFFECT OF PRE-TREATMENT WITH LIPOID SOLVENTS UPON THE SUBSEQUENT
RESISTANCE OF SEED COAT MEMBRANES FROM WISC. PED. 5-1 BARLEY
TESTED IN NORMAL TRICHLORACETIC ACID (25° ±1° C.)

(CHECK RESISTANCE, WITHOUT MEMBRANE, 31.2 OHMS)

TREATMENT	NO OF READINGS	READINGS IN OHMS RESISTANCE		
		LOW	HIGH	AVERAGE
Untreated................	24	900	1,900	1,368
Refluxed in ether 2 hours...	24	800	1,800	1,230
Refluxed in alcohol KOH for 2 hours...........	24	120	900	388

short period. These results led to testing the effect of pre-treatment in several fat solvents upon the subsequent membrane permeability. One lot of membranes from Wisc. Ped. 5-1 dry barley was refluxed for two hours in a solution of 2 per cent potassium hydroxide in 60 per cent alcohol; the membranes were washed thoroughly before testing. Another lot of the same membranes was refluxed for two hours in ethyl ether, hydrated through dilutions of alcohol, and then washed. These two lots and an untreated lot of the original sample were tested for their relative resistance in trichloracetic acid. The results are assembled in table V.

It is seen that the treatment in ether little alters the resistance of such membranes. The treatment in potassium hydroxide, however, causes a marked lowering of the resultant resistance. The solvent action of the alkali has evidently removed or altered the major por-

tion of the substances responsible for the relatively high resistance of these membranes. The fact that little of the substances making for the resistance were removed or altered by the ether treatment lends strength to the assumption that there is little true fat in the make-up of these semipermeable cutin membranes, or if so that it is not responsible for relative impermeability.

D. RELATIVE PERMEABILITY OF VARIOUS AREAS OF SEED ENVELOPE OF MAIZE

METHODS

The grain of *Zea mays* has been reported as possessing a semipermeable envelope in many respects similar to that of barley and wheat (21, 1, 17). The membranes taken from barley kernels being

TABLE VI

RESISTANCE OF SEED COAT MEMBRANES FROM DIFFERENT AREAS
OF KERNELS OF TWO VARIETIES OF MAIZE TESTED IN NORMAL
TRICHLORACETIC ACID AT 25° C.

(CHECK READING 40 OHMS)

VARIETY	PORTION TESTED	NO. OF READINGS	READINGS IN OHMS RESISTANCE		
			HIGH	LOW	AVERAGE
Yellow Dent.............	Top of kernel	25	660	410	515
Yellow Dent.............	Posterior	25	790	470	612
Yellow Dent.............	Portion over embryo	26	380	230	303
Blue Aleurone Cornell no. 633–13......	Anterior	24	460	280	368
Blue Aleurone Cornell no. 633–13......	Portion over embryo	28	220	247	176

rather small, it was decided to use the membranes from maize to determine the relative resistance of the various areas of the seed covering.

Kernels of Yellow Dent and Cornell no. 633–13 (Blue Aleurone), both in inbred lines, harvested just before complete hardening of the starch had resulted, were steeped for twelve hours in water at 32° C. The kernels were then cut into three portions: a top (apical) portion was cut off transversely and the remainder was split into a posterior

portion and an anterior portion containing the covering over the embryo. Membranes were prepared from these three portions, using the technique adopted for the preparation of the barley membranes, and their relative resistance to electrical conductivity was tested in normal trichloracetic acid. The data are assembled in table VI.

ORTON (17) has found that the rate of permeability of corn seed coat membranes to mercurial compounds in aqueous solutions varies with the variety of corn tested, and "apparently that portion of the seed coat covering the embryo side of the seed is more slowly permeable than that part covering the endosperm side." The results obtained in these experiments are in agreement with ORTON in part only. That there is a difference in degree of permeation among varieties is evident, but the data in table VII offer rather substantial evidence that the portion of the seed coat envelope covering the embryo is much more easily permeable (at least to this solution) than the covering of the endosperm, either over the apex or over the posterior surface (rachis side).

Discussion

A gradient of permeability to iodine in wheat grain coats was postulated by BRAUN (2) in disagreement with the conclusions arrived at by HARRINGTON and CROCKER (14). The latter, working with Johnson grass, had stated, "The solute enters only slightly through the surface of the grain but largely through a hilum or micropyle and spreads laterally and in a distal direction under the seed-coat." SCHROEDER (20) previously had suggested that absorption in wheat was exclusively at the embryo with the subsequent spread through the grain within the semipermeable envelope. COLLINS (7) also believed that only a small part of the water absorbed by the grain of barley entered by the general surface, special points of entry being in the germinal region. BEESKOW (1) and SHULL (21) found with maize that the diffusion of permeable solutes (iodine) through the membranes covering the endosperm is more rapid than any spread within. BROWN (5) found that the absorption of water by the seed of *Lolium perenne* is first in the germinal region, at the micropyle, and the slight upward spread within the coats causes a progressive swelling and stretching of the coats with a resultant in-

creased permeability at progressively higher levels. He later (6) related the same theories to the phenomena of absorption by the grain of wheat. Previous anatomical and morphological studies have shown that in each of the thus investigated Gramineae the seed is inclosed by a semipermeable cutinized (or suberized) envelope (7, 20, 21, 19, 16).

The present investigations have demonstrated that in barley grain the rate of absorption by the entire kernels can be correlated with the relative rates of permeability of the seed coats covering the anterior portion of the endosperm. These comparative relations have been demonstrated in solutions which permeate easily and in solutions permeating with difficulty. The anatomical investigations have shown a point of probable easy permeation, in agreement with COLLINS, SCHROEDER, and R. BROWN, as well as a gradient of thickness of the limiting membranes which is in support of the theories of HARRINGTON and CROCKER, and SHULL. In view of the results presented in this paper, it is entirely probable that not only is there an initial point of rapid permeation (the micropyle) but there is also a gradient of the seed coats to permeation, with the stretching and swelling resultant from the early basal absorption assisting in the progressive apicalward permeation and absorption by the grain. The thinness of the cutin membrane over the embryo, with the resultant decreased resistance of this area to permeation, probably accounts for the greater portion of the early absorption at the germinal end of the barley caryopsis.

The hull-less barleys studied have been found more easily permeable than the hulled varieties. Anatomical features which might be correlated with this increased degree are: thinner outer cutin membranes of the integument (of that portion covering the embryo in particular, see figs. 27–32); thinner membranes across the micropyle; and more areas of the integument in which there is less of the fatty substances in the inner row of cells. Although at times these variations may be partially controlling factors, it is thought more probable that differences in physical and chemical composition of the cutin membranes may be of more significant importance in regulating the degree of permeability. This hypothesis is substantiated by the increased rate of permeation induced by the wet environ-

ment during maturation, there being no alteration of anatomical structure in any of the varieties studied which could be correlated with these induced changes in permeability. It is then apparent that variations in degree of permeability are, at least in part, dependent upon the composition of the cutin membranes of the inner integument.

Summary

1. The developmental anatomy and morphology of the barley caryopsis has been discussed and illustrated.

2. The selective semipermeable envelope has been identified as the persistent crushed and cutinized inner integument together with the suberized, resistant tissue of the chalaza.

3. The inner integument has a thin inner cutin membrane and a much thicker outer cutin layer of variable thickness. The thickest portion is over the apex of the kernel near its attachment to the chalaza. It is also very thick over the flanks of the endosperm in the groove, is thicker laterally than anteriorly, and it is thicker apically than proximally. It is very thin over the embryo, its thinnest portion being the area immediately across the grouped cells at the micropyle. The thickness of these membranes was found to vary with the variety studied.

4. Relative permeability of the seed coats has been determined in two ways, by the rate of absorption from aqueous solution, and by the electrical conductivity of seed coat membranes in aqueous solutions of electrolytes.

5. With the use of these two differing methods it has been determined that: varieties exhibit wide differences in degree of permeability (the hulled varieties as a group being less permeable than the hull-less varieties); a wet environment during maturation induces a decreased resistance to permeation; and harvesting prematurely also induces a slightly decreased resistance to permeation.

6. The differences found in relative permeability could not be correlated definitely with any one existent variation in the anatomical structure of the semipermeable seed coat envelopes of the caryopsis. With some varieties, however, the heavier layer of cutin on the integument and the heavier deposits of the fat substance in the

integument cells seem to be correlated with an increased resistance to permeation.

7. The hull-less varieties investigated, as a group, were found more permeable than the hulled varieties but this difference was independent of the presence of the hulls.

The writer expresses his gratitude to Professor G. J. DICKSON, who suggested the problem and has offered much constructive criticism during the progress of the investigation; to Professors B. M. DUGGAR and ARTHUR J. EAMES, who have been most helpful; and to the National Research Council who awarded the Fellowship which facilitated the completion of the investigations.

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LITERATURE CITED

1. BEESKOW, H. C., The selective semi-permeability of the seed coats of corn. Univ. Chicago Library. 1924.
2. BRAUN, HARRY, A gradient of permeability to iodine in the wheat seed-coats. Jour. Agr. Res. 24:225–226. 1924.
3. BROWN, A. J., On the existence of a semipermeable membrane enclosing the seed of the Gramineae. Ann. Bot. 21:79–87. 1907.
4. ———, The selective permeability of the coverings of the seed of *Hordeum vulgare*. Proc. Roy. Soc. London B. 81:82–93. 1909.
5. BROWN, ROBERT, The absorption of the solute from aqueous solutions by the grain of wheat. Ann. Bot. 46:571–583. 1932.
6. ———, The absorption of water by seeds of *Lolium perenne* (L.) and certain other Gramineae. Ann. Appl. Biol. 18:559–573. 1931.
7. COLLINS, E. J., The structure of the integumentary system of the barley grain in its relation to localized water absorption and semipermeability. Ann. Bot. 32:381–414. 1918.
8. DENNY, F. E., Permeability of membranes as related to their composition. BOT. GAZ. 63:468–485. 1917.
9. ECKERSON, SOPHIA H., Microchemistry. Mimeographed outline.
10. GETMAN, F. H., and DANIELS, FARRINGTON, Outlines of theoretical chemistry. Wiley and Sons, New York. 1931.
11. GOLA, G., Ricerche sulla biologia e sulla fisologia dei semi a tegumento impermeabile. Mem. R. Acc. Sci. Torino II. 55:237–270. 1905.
12. ———, Ricerche sui rapporte tra i tegumenti seminali e le soluzioni saline. Ann. Botanica 3:59–100. 1905.

13. GUREWITSCH, A., Untersuchungen über die Permeabilität der Hülle des Weizenkorns. Jahrb. Wiss. Bot. 70:657–706. 1929.

14. HARRINGTON, G. T., and CROCKER, WM., Structure, physical characteristics and composition of the pericarp and the integuments of Johnson grass seed in relation to its physiology. Jour. Agr. Res. 23:193–222. 1923.

15. HAAS, P., and HILL, T. G., An introduction to the chemistry of plant products. Vols. 2. Longmans Green, London. 1921.

16. KRAUSS, LUISE, Entwicklungsgeschichte der Früchte von *Hordeum*, *Triticum*, *Bromus* und *Poa* mit besonderer Berücksichtigung ihrer Samenschalen. Jahrb. Wiss. Bot. 77:733–808. 1933.

17. ORTON, C. R., The permeability of the seed-coat of corn to mercury compounds. Phytopath. Abst. 17:51. 1927.

18. OSTERHOUT, W. J. V., The permeability of protoplasm to ions and the theory of antagonism. Science n.s. 35:112–115. 1912.

19. PUGH, GRACE W., and JOHAN, HELEN, Relation of the semipermeable membranes of the wheat kernel to infection by *Gibberella saubinetii*. Jour. Agr. Res. 45:609–626. 1932.

20. SCHROEDER, HEINRICH, Über die selective permeable Hülle des Weizenkornes. Flora 102: (N. F. 2) 186–208. 1911.

21. SHULL, C. A., and SHULL, S. P., Temperature coefficients of absorption in seeds of corn. BOT. GAZ. 18:212–226. 1924.

22. STILES, W., and JØRGENSEN, I., Quantitative measurement of permeability. BOT. GAZ. 65:526–534. 1918.

23. THARP, W. H., The influence of the maturation environment upon the permeability of the seed-coat of barley. Amer. Jour. Bot. 19:836. 1932.

24. TRUE, R. H., On the development of the caryopsis. BOT. GAZ. 18:212–226. 1893.

ECOLOGICAL STUDIES ON THE HIGH PLATEAUS OF UTAH

HELEN DIXON

(WITH THREE FIGURES)

Introduction

The region studied is located mainly in Wayne County, Utah, but it includes parts of Sevier County (in the vicinity of Fish Lake) and of Garfield County (Boulder Mt. on Aquarius Plateau). It lies within a quadrangle extending from 111° to 112° west longitude and from 38° to 38° 45′ north latitude. The altitudinal range is from 5250 feet at Notom to 11,600 feet on the tops of the High Plateaus.

The drainage is into the Colorado River by way of the Fremont River. Physiographically the region belongs to the province designated by the Powell Survey (3) as "the High Plateaus of Utah." Because of its sparse population and its inaccessibility to the ordinary traveler, it is comparatively unknown and is still unspoiled by tourists.

Geology and topography

The geology was first worked out in 1879 by Capt. C. E. Dutton of the Powell Survey (3). Little scientific work has been done in this territory since then. The Plateau Province of Powell has been above the sea since the close of the Cretaceous. Tertiary lacustrine deposits and lava flows have covered the earlier marine deposits. The present altitude of over 11,000 feet is due mainly to subsequent faulting and uplift.

The highlands considered in this report comprise the southern half of the easternmost of the three belts of Powell's High Plateaus. They include the Fish Lake Plateau with Mts. Marvine, Terrill, and Hilgard, its outliers northeast of it, the Awapa Plateau, Thousand Lake Mt., and the Aquarius Plateau.

Fish Lake Plateau is a small tableland 10–15 miles long by 2–5 miles wide. It reaches a maximum altitude of 11,600 feet. Except

along its southwest margin where it merges with the Awapa Plateau, it is bounded by precipitous lava walls such as the Grass Valley fault-scarp, a 4300 foot cliff on the northwest, and the 2700 foot cliff above the lake on the southeast side.

There is evidence of glaciation during the Pleistocene. The location of the terminal moraines at 9000 feet in Fish Lake Basin and in Summit and Moraine valleys, however, disproves any glacial origin for the lake basin itself, the lake level being 8750 feet. DUTTON (3) found evidence of a former outlet into the Great Basin at the south end of the lake. In the process of uplifting, the basin was so tilted that the drainage was reversed. The water of Fish Lake now joins that of Seven Mile Creek at the north end, and together they flow through the Fremont Reservoir into the headwaters of the Fremont River and thence into the Colorado River.

Fish Lake is a deep body of water 6 miles long and nearly 2 miles wide. Its west wall rises steeply 2600 to 2700 feet to the top of the plateau. The east wall is even more abrupt but not so high, only 1700 feet. Just over its crest are the two Crater Lakes.

The Awapa Plateau adjoining Fish Lake Plateau on the southeast was uplifted with the latter along the Grass Valley fault. Its western front stands 2000 feet above Grass Valley. Awapa Plateau has an area of about 700 square miles. Its surface is a rocky lava slope descending from 9000 feet at the northwest, west, and southwest rims to Rabbit Valley, a crescent shaped depression lying at 7000 feet above the sea. The Fremont River flows through this valley after its descent from the Fish Lake Highlands and deposits here much of its load, forming a rich alluvial plain.

Rising above Rabbit Valley along the concave side of the crescent is Thousand Lake Mt. It too is a remnant of erosion, having been separated from the Aquarius Plateau by the work of the Fremont River and its tributaries. Thousand Lake Mt. is an even smaller table than Fish Lake Plateau, its nearly level lava-capped summit being about 5 miles long and not quite 2 miles wide. To the east and southeast is the great rock desert of the Water Pocket Flexure, while to the south across the Fremont Valley is the Aquarius Plateau.

At the lower end of Rabbit Valley, the Fremont River passes through the Red Gate into a series of canyons and gorges cut

through 2500 feet of brilliantly colored sediments on its 100 mile course to the Colorado River.

High above all of this is the Aquarius Plateau, which lies within the Powell National Forest, the largest forest in Utah. This plateau is roughly L-shaped, 35 miles long by 10–18 miles wide. Its altitude ranges from 10,500 feet on the south and west to 11,600 feet on the east, where it stands 5500 to 6000 feet above the rock desert at its base. The lava cap varies from 1000 to 2000 feet in thickness. Over three-fourths of its margin is bounded by massive cliffs at the bases of which are steep talus slopes. Numerous glacial lakes, cirques, and moraines furnish evidence that the summit and upper slopes have been glaciated.

Both the Water Pocket Flexure and Miner's Mt. are subsidiary domes extending southeast from below the Red Gate and rising to 8250 feet. They have been deeply and intricately dissected by narrow gorges and canyons antecedent to their uplift. Most of the gorges are dry except during a rain; but cloudbursts occasionally bring a head of water 15 to 20 feet high rushing through the washes, destroying all in its path.

The Water Pocket Flexure has been of interest to the mineralogist because of its deposits of copper and uranium; to the paleontologist because of the abundant Triassic material, and to the anthropologist because of the interesting remains of the Fremont culture of the cliff-dwellers found in its canyon walls. Botanically it is of interest as a northern outpost of the Lower Sonoran desert vegetation.

Climate

The latitudinal extent of this area is only three-fourths of a degree, but its altitudinal range is 6000 feet. Figures from the records of the United States Weather Bureau (1) corroborate SAMPSON'S (5) statement that climatic differences due to altitude are intensified by the type of relief features peculiar to Utah. The length of the growing season, the temperature, precipitation, rate of evaporation, wind, all of these vary greatly even in as small an area as that under consideration. In the lowlands the growing season is two or three times as long (180 to 200 days) as on the tops of the plateaus (60 to 90 days). This factor alone would show its effects on the plant cover.

PRECIPITATION.—Although dependent somewhat upon altitude, precipitation is more affected by topography. Loa, at 7000 feet, has less precipitation than St. George at 2880 feet. BOWMAN (2) remarks that most of the High Plateaus are as dry as southwestern Arizona at one-half the altitude. DUTTON (3) and SAMPSON (5) point out that all lands below 7000 feet in the plateau country are deserts, receiving less than 8 inches of precipitation per year.

The zones lying above 7000 feet receive more moisture than the lowland stations. BOWMAN (2) estimates that the Aquarius Plateau receives 24–30 inches. Fish Lake Plateau and Thousand Lake Mt. probably get as much, for their vegetation is similar. SAMPSON gives figures for three of the forest zones. The aspen-Douglas fir zone is the wettest, receiving 25–29 inches per year. The Engelmann spruce zone comes next with 25.9 inches. The western yellow pine zone has an average of only 19 inches. No figures are given for the piñon-cedar zone, but it probably gets about 15 inches per year. SAMPSON found the average monthly precipitation during the summer to be as follows:

> Engelmann spruce zone . . . .   2.16 inches
> Douglas fir-aspen zone . . . .   2.44 inches
> Western yellow pine zone . . . .   1.52 inches

EVAPORATION.—Owing to their higher temperature and lower humidity, as well as to their scantier precipitation, the lowest altitudes have the highest rates of evaporation. There is a gradual decrease up to the aspen zone, where it reaches a minimum. In spite of its lower. temperature, there is an increase in the spruce zone over that of the zone below. This is due not only to lower precipitation, but also to higher winds. During the growing season, the aspen-Douglas fir zone has a total wind of 12,500 miles, compared with that of 27,500 miles in the Engelmann spruce zone (9).

Soils

The soils of the region vary widely in their physical and chemical composition. Residual lava soils occur chiefly on the tops of the High Plateaus, and also on the entire surface of the Awapa Plateau. They are usually thin and stony, owing to the resistant character of

the parent rock. Their moisture content therefore is low and their air content high.

Residual sands, gravels, and clays are found below the lavas on all of the plateaus. Sands and clays are most abundant and show contrasting vegetation in the same climatic zone.

Residual limy soils are less common. On the northeast face of Aquarius Plateau at about 7500 feet altitude is a spur of richly calcareous soil derived from gypsum. Growing here, as at Bryce Canyon where the soil is from a very calcareous sandstone, is *Pinus aristata*.

Residual organic soils accumulate best in poorly drained depressions and usually support a meadow vegetation. A vein of peat 2–3 feet below the surface of glacial till in the *Pinus ponderosa* forest indicates a former climate somewhat different from that of the present. Humus layers cover other soils on all vegetated surfaces.

Aeolian soils in the form of dunes are piled against the White Cliff domes on the sandstone ledges and on a miniature scale on the floodplain of the Fremont River. These soils are like the residual sands except that they are less stable.

Alluvial soils are largely under cultivation. They occur most plentifully in Rabbit Valley and in the Fremont Valley at Fruita. Slope wash has helped to fill Summit Valley and the Fish Lake basin. Alluvium is usually deep and level and better watered than surrounding areas. Its vegetation is therefore distinct.

Lacustrine soils are found along the shores of shallow lakes. Like stream-borne soils, they are level, deep, and well watered. In addition they are usually fine and richer in humus although their drainage is poorer.

Glacial drift is restricted to levels above 9000 feet at Fish Lake and perhaps on the other plateaus. Terminal moraines exist at this level.

Talus, because of its coarseness, usually supports only pioneer stages of vegetation. In the montane zone such soil more frequently develops a conifer rather than an aspen forest. Perhaps the crevices where seeds may start are too dark for aspens.

Most of the soils were circum-neutral (pH 6.8–7.2). The meadow soils showed pH 6.8 or above; soil from the Atriplex desert at Notom

tested pH 7.8–8.0; while the mud flats on the shores of the Crater Lakes at 9750 feet had a pH of 7.3.

Vegetation

The various plant zones, like the belts of climate, are more or less dovetailed together. The southern limit of this area seems to be drawn on the tension line between the upper and lower Sonoran zones, for in the arid regions beyond the Red Gate such conditions exist as permit the advance of the vanguard of the lower Sonoran flora into territory which theoretically should be northern desert.

FIG. 1.—Alkali desert below Notom showing greasewood-shadscale association; *Populus fremontii* along the wash. Photograph by S. B. Spira.

Only a few of the southern desert forms have succeeded in climbing over the rim into the Great Basin. The desert areas on the basin side of the divide are occupied by the northern desert formation dominated by *Artemisia tridentata*. Both of these formations are replaced, at least temporarily, on alkali soils by *Sarcobatus vermiculatus* and its associates (fig. 1). Above the desert, in the cooler, less arid zone extending from 6000 to 7500 feet, are extremely open stands of scrub timber. Near Fruita this zone is occupied by the southern semidesert formation consisting chiefly of *Pinus edulis* and *Juniperus utahensis*, with some northern desert species. The cedar-piñon association forms a belt also above the *Artemisia tridentata* zone on

Thousand Lake Mt., Aquarius Plateau, and the extreme rim of the Awapa Plateau above 8000 feet.

The northern semidesert is likewise characterized by the cedar; but instead of by piñon, it is accompanied by *Cercocarpus*. It separates the sagebrush desert from the montane forest on the west face of Fish Lake Plateau.

There are two other associations between the desert and the forest which are probably only subclimaxes here. In some places are nearly pure stands of *Quercus utahensis*. In others a chaparral consisting of *Arctostaphylos platyphylla* is conspicuous. The composition of the scrub forest zone seems to vary somewhat with edaphic conditions.

Where the rainfall is 15 to 20 inches, the slopes not too steep, and the soil well drained, open but practically pure stands of *Pinus ponderosa* occur. These are the first true forests. The trees are tall and straight and not so scattered as in the semidesert. They form the climax association on the northern and eastern slopes of the Aquarius Plateau from 7500 to 9000 feet, and at about 8000 feet on some of the sandy spurs on the east face of Thousand Lake Mt. Isolated groups occur as low as 5000 feet in favorable situations on Miner's Mt. and the Water Pocket Flexure. There are no western yellow pines on either Fish Lake or the Awapa Plateaus.

Above the western yellow pine forest occurs the most mesophytic plant association in all Utah, the montane forest. In this region it is best developed from about 8700 to 9500 feet, but may extend far down along mountain streams, even into the piñon zone. The dominant trees are *Pseudotsuga mucronata* and *Populus aurea*. Mixed with them are *Abies concolor* and *Picea pungens*. This formation is well represented on all three of the High Plateaus.

The subalpine forest consists mainly of Engelmann spruce; but in places *Abies lasiocarpa* is abundant. Both aspens and Douglas firs extend well up into this zone. The main stand of the subalpine forest lies between 9500 feet and 11,000 feet on these plateaus. It extends down to 9000 feet in canyons and exists only as scattered bunch forests beyond 11,000 feet.

The alpine meadow formation occupies valleys and depressions at the subalpine level. *Caltha rotundifolia, Polygonum bistortoides,*

Phleum alpinum, and *Pedicularis groenlandica* seem to be constant members of this association. The alpine meadow may represent a permanent climax or a long-time temporary climax. Whichever may be the case, it is a characteristic feature of the landscape in the subalpine zone.

In the open spaces on the summits and high ridges the soil is thin and stony, and because of its good drainage and exposure to high winds, it is dry. Here an alpine scrub made up of prostrate shrubs and minute mat-forming herbs develops. This alpine-desert climax is found on the tops of all the High Plateaus and on the summits of Mts. Marvine and Terrill. It corresponds to the dry tundra of the arctic.

1. SOUTHERN DESERT ZONE

DESERT CANYONS OF WATER POCKET FLEXURE.—Although the upland near Fruita belongs to the piñon zone, the canyons and washes here exhibit a more southern flora, representing the northern extension of the Lower Sonoran. This may be accounted for partly by their lower altitude, but it is due also to the fact that the water courses of the Colorado River system must serve as the highways of migration for the Lower Sonoran flora, since overland passage to the north is blocked by the high plateaus in Arizona. In some respects these canyons in the Capitol Reef resemble those in Zion National Park. Both are carved into the Vermilion and White Cliff sandstones; Zion Canyon is farther south and about 2000 feet lower, and so is warmer and has a longer growing season. In spite of this its climate is less arid than that of the Water Pocket canyons, perhaps because it has been eroded deeper and so is more shaded. However, since the Fremont Valley (fig. 2) is farther from the centers of distribution of Lower Sonoran species, it has fewer representatives from that zone.

Most of the canyons in the Water Pocket Flexure are deep and very narrow. All of them except the Fremont itself and several of its larger tributaries are usually dry. Floodplain studies therefore were confined to the lower end of Spring Canyon, to Pleasant Creek, and to the Fremont River. The last is heavily charged with red silt, but its current is so rapid that most of this is carried on to the Colo-

rado River and only the heavier sand and gravel are deposited, except in a few places behind bars or levees.

Mats of *Halerpestes cymbalaria* seem to have been the first pioneers on the wet sand of floodplains. *Ranunculus* spp. and *Muhlenbergia asperifolia* soon follow. On the dry sand of levees and small dunes, *Abronia elliptica* and *Franseria acanthicarpa* are succeeded by *Salsola pestifer*, *Cenchrus pauciflora*, and *Atriplex canescens*. *Salix lasiandra* is the most common pioneer streamside shrub. *Salix exigua* and other willows may accompany it. *Asclepias incarnata*, *Melilotus alba*, *Cleome lutea*, *Stanleya integrifolia*, *Artemisia tridentata*, and *Chrysothamnus graveolens* grow quite tall along the streams. *Clematis ligusticifolia* festoons many of the streamside shrubs. Each zone seems to have its own species of rose. Here it is *Rosa puberulenta*. In sunny places on sandy floodplains *Populus fremontii* follows the willows, although it is sometimes the pioneer tree, as on the floodplain of Pleasant Creek in the desert below Notom (fig. 1). Above the Water Pocket Flexure along the same stream *Populus trichocarpa* and escapes of *P. alba* are common.

On shadier floodplains, *Acer interior* replaces the Fremont cottonwood. With it is *Populus angustifolia* and sometimes *Toxicodendron rydbergii*. In the still denser shade of narrow canyons *Cornus stolonifera* and *Betula fontinalis* grow on the banks of the streams. These have come down stream from a zone or two above. *Fraxinus anomala* and *Rhus utahensis* usually occur abundantly back from the water's edge on the higher floodplain.

As the canyon is widened and the floodplain built up, the former streamside zone becomes more arid, both as to soil and to atmosphere. Its vegetation then retrogresses to the desert formation which is the climax of the region. So on the higher grounds of wide floodplains various cacti (*Opuntia* spp., *Echinocactus*, *Echinocereus*), *Ephedra viridis*, *Yucca harrimaniae*, *Lepargyrea rotundifolia*, *Wyethia scabra*, *Coleosanthus microphyllus*, *Chrysopsis* spp., and the desert grasses *Hilaria jamesii* and *Sitanion hystrix* are typical. Wherever the dry sand is piled up into dunes, *Abronias* and their associates come in.

GRAVEL WASHES.—In the gravel washes where the soil is moister, deeper, and more level than that on the uplands, the vegetation presents a more desert-like aspect than that of the ridges above.
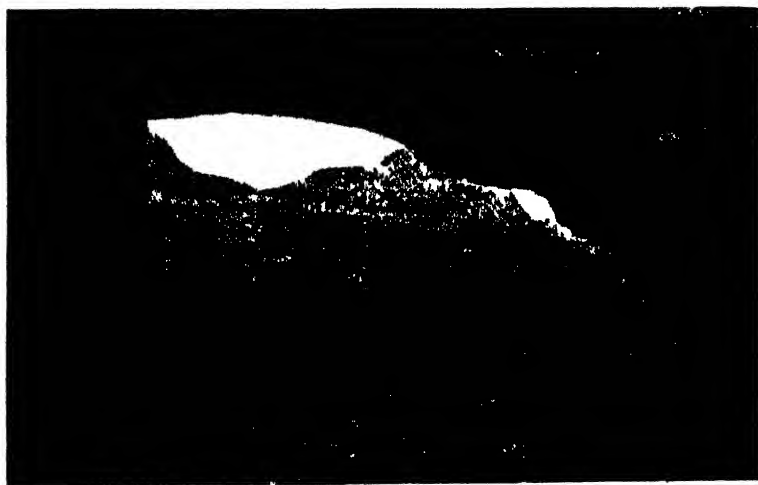
Fɪɢ 2

Fɪɢ 3

Fɪɢs 2, 3 —Fig 2 (above), Fremont valley above Fruita The alluvial soil is irri-
gated for cultivation The southern desert formation occupies the lower slopes, the
cedar-piñon woodland, the upper slopes. Fig 3 (below), through the arch of the
Wayne County (Utah) natural bridge Cedar-piñon climax on talus of White Cliff
sandstone.

Although they are usually dry, a cloudburst every few years is suffi-
cient to sweep the washes clean of all vegetation. Shrubs and herbs
may return in the time intervening between wet years, but not such
slow growing forms as cedars and piñons. In the draws are *Cleome
lutea*, *Polygala subspinosa* (?), *Lavauxia howardii*, *Sphaeralcea
grossulariaefolia*, *Phacelia corrugata*, *Delphinium scaposum*, *Astra-
galus thompsonae*, *Chamaesyce fendleri*, *Asclepias cryptoceras*, *Erio-
gonum alatum*, and several cacti. Shrubby forms such as *Ephedra
viridis*, *Cowania stansburiana*, *Odostemon fremontii*, *Amelanchier
utahensis*, *Pleiacanthus spinosus*, *Rhus utahensis*, *Artemisia triden-
tata*, and *Chrysothamnus graveolens* follow the herbs. In shady places
Quamoclidion froebelii, *Pentstemon caespitosus*, *Datura meteloides*,
Asclepias labriformis, *Artemisia filifolia*, *Jonesiella asclepiadoides*,
and others are found instead.

ROCK CANYONS.—Higher upstream the sandy deposits are limited
to deltas or bars at the mouths of side canyons. Vegetation on the
rock floors differs from that of the floodplains. In shady places
Odostemon fremontii is common. *Juniperus utahensis*, *Pinus edulis*,
and *Cercocarpus intricatus* are occasionally seen on the ledges of the
canyon walls. On sunny rocks *Ephedra torreyana*, *Lepargyrea ro-
tundifolia*, and a shrubby *Eriogonum* appear. Near a spring at the
mouth of a side canyon is a clump of *Celtis rugulosa*.

Narrow canyons develop more mesophytic vegetation. Both west-
ern yellow pine and Douglas fir, with a rank undergrowth of
Odostemon repens, *Ceanothus fendleri*, *Amelanchier utahensis*, *Pent-
stemon eastwoodii* var. *undosus*, *Selaginella mutica*, and *Phlox austro-
montana* are found. A colony of *Pinus ponderosa* accompanied by a
thicket of *Arctostaphylos platyphylla* occurs on a north-facing sand-
stone cliff in the lower piñon-cedar zone. The presence of crustose
and foliose lichens and xerophytic mosses on white sandstone indi-
cates that erosion is slow.

2. PIÑON ZONE (SOUTHERN SEMIDESERT ZONE)

The nut pine-cedar association is the climax of the southern semi-
desert zone (fig. 3). It is reached mainly through a xerarch or occa-
sionally through an alkali succession, for there are no ponds or lakes,
and the stream beds at this level are dry except when it rains. Stabil-

ity and depth of soil are the main factors in the xerarch succession in this zone, for even in the climax pine-cedar woodland the plants are spaced so far apart that light and moisture conditions are not very different from those of the pioneer stages. The tree stages, however, do require a somewhat deeper soil. The type of soil does not seem to be important, for this southern semidesert formation occurs on white sands, red stony clays, and on heterogeneous soils derived from conglomerates containing huge black lava boulders. *Acarospora* and other crustose lichens initiate the succession on the lava. They are followed by *Parmelia* spp. and the moss *Tortula muralis*. Shale and sandstone weather so rapidly that these earliest stages are usually omitted on them. Crevice plants such as *Selaginella mutica* are more commonly the first pioneers on the sandstone ledges. In the deeper crevices almost any species of the piñon-cedar association, including the dominant trees themselves, may be found. Pioneer crevice herbs aid in enlarging the small pockets and accumulating sufficient soil for shrubs and eventually for trees. The wind, too, is an important agent in this succession, for it honeycombs the sandstone walls (fig. 3) and then piles the sand on the ledges in the form of dunes. On these dunes the pioneers are of a different sort. They must have root systems which can aid in binding the shifting sand or which can endure a fluctuating soil level. *Abronia elliptica, Franseria acanthicarpa, Salsola pestifer, Arenaria aculeata, Townsendia arizonica, Hilaria jamesii, Erigeron argentatus, Gilia dixonae* Nels., *Phlox canescens,* and several species of cacti are dune pioneers. *Odostemon fremontii* and *Rhus utahensis* represent the shrub stage. The trees are less common on dunes than in large crevices or in rocky soils where anchorage is better.

The herbs and shrubs are much more abundant and the trees are closer together, larger, and more symmetrical on coarse rocky soils than on the wind-polished sandstone plateaus (fig. 3). The dominant herbs are *Sphaeralcea grossulariaefolia, Lygodesmia juncea, Calochortus nuttallii, Lavauxia brachycarpa, Stipa speciosa, Aristida fendleriana, Andropogon scoparius, Artemisia wrightii, Pentstemon ophianthus, Pleiacanthus spinosus, Opuntia* spp., *Echinocactus xeranthemoides, Echinocereus triglochidiatus,* and *E. mojavensis.* The woodier forms include *Yucca harrimaniae, Artemisia tridentata, Chrysotham-*

nus graveolens, *Lepargyrea rotundifolia*, and *Amelanchier utahensis*.
Philadelphus microphyllus, *Odostemon fremontii*, *Rhus utahensis*, and
Quamoclidion froebelii are more common on shady slopes. These
species perhaps precede the trees, but continue on with them in the
richest piñon-cedar woodland.

STREAMSIDE VEGETATION IN PIÑON ZONE.—The permanent
streams in, this zone are often fringed merely with more luxuriant
sagebrush and rabbit brush, but the narrow-leaf cottonwood seems
to be the typical streamside species. It may be preceded by willows.
Rosa puberulenta and *Rhus utahensis* accompany it. *Urtica breweri*
is common on the floodplain of the Fremont River. *Acer interior* and
Betula fontinalis replace *Populus angustifolia* in shaded places.
Picea excelsa is seen along the streams in the upper limits of the zone.

3. ALKALI ASSOCIATION

On shaly soils alkali crusts may form, for drainage is poor and the
salts are not leached out. As at Notom where the pH is 7.8 to 8.0,
the piñon-cedar woodland is replaced indefinitely by a long-time
temporary climax of *Atriplex confertifolia* and *Sarcobatus vermicula-
tus*, which may be introduced by *Distichlis spicata* on flats of alkaline
mud along the stream. The alkali desert formation (fig. 1) covers the
soil very scantily; vast stretches are plantless.

4. NORTHERN DESERT CLIMAX

Almost the entire Awapa Plateau, the west side of Aquarius
Plateau, and the lower slopes of the Fish Lake Plateau are covered
by the sagebrush formation. The successions here, too, are all
xerarch, since there are no ponds, lakes, nor even streams. The lava
rock shows the usual pioneer lichen and moss stages. Xerophytic
herbs are first in the crevices. They are soon accompanied down at
7000 feet by cacti and yucca, but up at 9000 feet by *Gilia aggregata*,
Pentstemon strictus, *Calochortus nuttallii*, *Eriogonum piperi*, a bright
rose-colored *Opuntia*, and *Lupinus* spp. When the soil layer is deep
enough, *Artemisia tridentata* and *Tetradymia canescens inermis* be-
come dominant, but the herbs continue in the interspaces.

Swamps in the sagebrush zone between Richfield and Fish Lake
are of the sedge type. Anderson Wash southeast of Sigurd is fringed

with *Salix* spp., *Acer negundo*, *Prunus melanocarpa*, and *Populus angustifolia*. If swamps and streams existed in the sagebrush desert on the Awapa Plateau they would probably have similar vegetation.

5. NORTHERN SEMIDESERT

The cedar-mountain mahogany climax occurs on the middle slopes of the west face of the Fish Lake Plateau from about 8000 feet altitude to over 9000 feet on south facing walls. Piñons are dropping out and with them the southern semidesert shrubs, *Lepargyrea rotundifolia*, *Rhus utahensis*, *Fallugia paradoxa*, and *Cowania neomexicana*. Instead of these, *Juniperus utahensis* is accompanied by *Cercocarpus parvifolius*, *C. ledifolius*, *Juniperus scopulorum*, and *Quercus utahensis*, in a mixed woodland in which grow *Artemisia tridentata*, *Purshia tridentata*, *Prunus melanocarpa*, *Amelanchier oreophila*, *Sericotheca diffusa*, *S. microphylla* (?), *Eriogonum effusum*, *Pachystima myrsinites*, and species of *Symphoricarpos* with a rich herbaceous complement of *Lupinus*, species of *Eriogonum*, *Castilleja*, *Pentstemon*, *Solidago bigelovii*, *Gutierrezia*, *Artemisia frigida*, *Agropyron spicatum*, and others. In the lower part of this mountain mahogany zone are *Yucca* and *Opuntia*.

The distribution of these four desert formations seems to be determined by several factors. Temperature may limit the range of the southern desert species. They are most common below 6000 feet in the Fremont Basin. Those requiring long hot summers and mild winters have not yet migrated to the 9000 foot crest of the Awapa Plateau, and so are not included in the desert flora on the Great Basin side of the divide. The southern semidesert occurs in a rather definite belt between 6000 and 7500 feet on the sandstone plateaus, but on lava soils the sagebrush formation occupies this level, and the cedar-piñon zone is pushed up to even 9000 feet in some places. Hence competition, especially with *Artemisia tridentata*, seems to be more important than temperature in limiting the range of cedars and piñons, for prostrate forms of both were found in the subalpine zone on Thousand Lake Mt.

6. PINUS PONDEROSA CLIMAX

In the western yellow pine zone, studies were made of the upland xerarch succession and of the streamside vegetation. The only hy-

drarch succession found was on the tension line between this and the aspen zone. Since the climate in depressions is cooler than that on adjacent ridges, it is included in the account of the aspen rather than in that of the western yellow pine zone.

TIDESTROM'S map (6) shows *Pinus ponderosa* between 7000 and 9000 feet. SAMPSON (5), however, gives its range as 6200 to 7600 feet. On the Aquarius Plateau the main forest lies between 7500 and 9000 feet on the east face and 7000 and 8500 feet on the north face, with isolated colonies as low as 5600 feet. The western yellow pines on the east spurs of Thousand Lake Mt. are at an altitude of about 8000 feet. *Pinus ponderosa* seems to occur here on flat areas of both residual and aeolian sands and on steep slopes of glacial drift. Sandy slopes may be too dry and flat clays too poorly drained for it.

XERARCH SUCCESSION.—On resistant rock, crustose lichens form the initial stages. These are followed by *Parmelia* and other foliose forms, and by xerophytic mosses. After these pioneers, together with agents of erosion, have disintegrated the rock, xerophytic herbs such as *Antennaria aprica, Pediocactus simpsonii, Opuntia fragilis* (and other *Opuntia* spp. in the lower parts of the zone), *Eriogonum racemosum, Bouteloua gracilis, Sitanion hystrix*, and *Oreocarya suffruticosa* come in on the new soil. In sandy places *Abronia elliptica, Phlox stansburyi*, and *Pentstemon arenicola* are common pioneers. *Pentstemon cyananthus* (?), *Gilia aggregata, Linum lewisii*, and *Oreocarya argentea* follow them. *Rosa* spp. are common in loose sand, and *Cercocarpus intricatus* in crevices of sandstone cliffs. In the lower part of the zone *Yucca*, several cacti, and *Pleiacanthus spinosus*, found in the piñon zone, are common pioneers, especially on rocky soils.

Purshia tridentata, Ceanothus velutinus, C. fendleri, Amelanchier alnifolia, Sambucus melanocarpa, Cercocarpus montanus, Symphoricarpos oreophilus, and species of *Ribes, Grossularia*, and *Rosa* are fairly common representatives in the shrub stage throughout the zone. *Artemisia tridentata* and *Chrysothamnus graveolens* come in on deeper soils. In the lower part of the zone, *Cowania neomexicana* and *Fallugia paradoxa* extend up from the piñon zone; and in the upper part are such shrubs as *Odostemon repens, Arctostaphylos uva-ursi*, and *Prunus melanocarpa*, all common to the aspen zone. Thickets of

Arctostaphylos platyphylla or of *Quercus utahensis* are sometimes seen on sunny slopes. Occasional specimens of *Pinus edulis* or of *Juniperus utahensis* grow taller and straighter here than in their own zone, another indication that competition may be a limiting factor determining their range. *Juniperus scopulorum* is frequent as an understory tree in the western yellow pine forest. Seedlings of *Picea pungens* occasionally come up on open hillsides. The western yellow pine itself comes in when the soil is deep enough and is well drained. It reproduces well, as is shown by the large number (38) of vigorous seedlings in a 10 meter quadrat which had been protected from grazing for five years. In a quadrat of equal size and similar situation but grazed were only nine pine seedlings. Overgrazing leads to soil erosion, and *Pinus ponderosa* seems to require deep soil.

Since this forest is an open one, many of the pioneer forms continue as undergrowth in the climax stage. Certain species, however, are especially characteristic under the pines. *Peltigera canina* grows on the fallen needles. *Phlox austromontana*, a prostrate, awl-leaved, mat-type species with white to clear pink flowers, is perhaps the most conspicuous herb. *Tithymalus luridus, Pseudocymopterus montanus, Antennaria aprica*, and *Erigeron vetensis* are also common. *Poa pratensis* and *Agropyron spicatum* are dominant grasses. *Clematis pseudoalpina* grows at the bases of tree trunks. On the north face of Aquarius Plateau *Castilleja linariaefolia, Geranium richardsonii, Hymenoxys odorata, Lupinus lepidus*, and a red *Pentstemon* are abundant. *Senecio* and *Hymenoxys* are considered by the rangers as indicative of overgrazing. In places the sheep have practically eliminated the shrubs in the pine forest.

STREAMSIDE VEGETATION OF PINUS PONDEROSA ZONE.—*Salix subcoerulea* and *S. bebbiana* fringe the streams at 8000 feet. The first tree on the floodplain is *Populus angustifolia. Betula fontinalis* is a close second. With them on wet banks are gooseberry, elderberry, currant, and rose, accompanied by *Veronica arvensis, Juncus* spp., *Artemisia racemosa, Montia chamissoi, Equisetum hiemale*, mosses, and liverworts. Growing in the stream among rocks are *Cardamine infausta* and *Catabrosa aquatica*. In the next belt back from the stream is *Picea pungens* with *Juniperus scopulorum* and *J. sibirica*. Among these conifers are *Smilacina stellata, Geranium richardsonii*,

Arenaria eastwoodii, and *Wyethia mollis*. The willows and cotton-woods seem to be limited to the stream's edge, but the blue spruce has a wider range.

OAK THICKETS.—SAMPSON (5) points out the oak as an ecological equivalent of *Pinus ponderosa* which is capable of replacing the pine. Usually the sheep browse the oak so closely that it remains a low shrub. On steep, rocky, more inaccessible slopes it attains a height of 7–10 feet. Typical pine forest pioneers seem to precede *Quercus utahensis* and a typical pine undergrowth accompanies it: *Odostemon repens, Castilleja linariaefolia, Phlox austromontana, Ceanothus fend-leri*, and seedlings of *Juniperus scopulorum*. On Aquarius Plateau the oak association seems to be a pioneer tree stage which precedes the climax western yellow pine forest, and apparently is not an ecological equivalent of it. On the west face of Fish Lake Plateau the oak appears on ridges in the upper part of the mountain mahogany zone at the same level where aspens are first seen in the ravines.

MANZANITA.—*Arctostaphylos platyphylla* also forms only small local colonies here, and so can scarcely be considered as anywhere replacing the pine as a climax form. Instead it seems to occur as a subclimax stage in the xerarch succession to the pine forest.

FOX-TAIL PINE.—Although classified by TIDESTROM as belonging in the aspen-spruce zone, *Pinus aristata* is found here only in two small colonies in the western yellow pine zone on the north face of Aquarius Plateau. In such a situation it may be a relict of the Pleistocene.

7. MONTANE ZONE

This zone is best exhibited on north and east facing slopes in a belt extending from 8700 to 9500 feet in altitude. The aspen forms extensive groves. The Douglas fir is not found in as pure stands as the aspen; it seems to be restricted to the steeper, more exposed slopes, rocky ridges, talus slides, etc., while the aspen occupies morainic lacustrine, or alluvial deposits where the soil is deeper and moist. In shaded mesophytic canyons and about springs on slopes, aspens descend to 7500 feet, and perhaps occasionally lower. The lowest altitude recorded for Douglas fir in this region is about 6000 feet. On the other hand, both species occur mixed with Engelmann spruce and subalpine fir in the forests below the lava caps on all three of the

plateaus. There are dwarf aspens as high as 10,700 feet. As a rule, however, they do not reach the summits.

Like the sagebrush, the aspen takes the deeper, moister soils of pockets and shady north faces in the lower part of its range, but comes out on the ridges in the upper stretches, for below, the ridges are too dry, and above, the summers in the pockets are too short.

The Douglas fir association seems usually to climax a xerarch succession, but both xerarch and hydrarch series may terminate in the aspen association.

XERARCH SUCCESSION TO CLIMAX MONTANE FOREST.—Wherever resistant rock is found, the earliest stages are the usual crustose lichens, *Acarospora bella*, *A.* spp., *Caloplaca* spp., and *Candelariella vitellina*, followed by *Parmelia molliuscula*, *P. conspersa*, and the moss *Tortula muralis*. The succession on soil begins with xerophytic herbs, the most conspicuous of which are *Pleiacanthus spinosus*, *Artemisia frigida*, *Chaenactis douglasii*, and *Pediocactus simpsonii*, this last a cactus which seems to be limited to moderately high altitudes (8700–9800 feet) and to thin rocky soils. Other xerophytes accompany these pioneers later and eventually crowd them out: *Erigeron bloomeri*, *E. flagellaris*, *Antennaria aprica*, *Eriogonum racemosum*, *E. jamesii*, *Castilleja exilis* and other species, *Lupinus argenteus*, *L. alpestris*, *Sitanion hystrix*, *Stipa comata intermedia*, *Koeleria cristata*, *Gilia aggregata*, *Phlox cortezana*, *Senecio multilobatus*, *S. mutabilis*, and others. These herbaceous xerophytes form the present cover on the dry knolls of the spurs projecting from the façades of the plateaus. Below them is a shrub zone which dovetails into the aspen grove or Douglas fir forest, as the case may be. The xerophytic shrub pioneers are common to the pine forest below. They include *Tetradymia canescens inermis*, *Artemisia tridentata*, *Purshia tridentata*, species of *Chrysothamnus*, *Amelanchier alnifolia*, *Sambucus melanocarpa*, *Rosa fendleri*, *Ceanothus fendleri*, *Ribes leptanthum*, *R. viscosissimum* (?), and *Prunus melanocarpa*. Just preceding the aspen is *Symphoricarpos oreophilus*, which remains as the dominant undershrub in the aspen grove. *Lepargyrea argentea*, *Odostemon repens*, *Lonicera involucrata*, *Arctostaphylos uva-ursi*, and *Pachystima myrsinites* form the undergrowth in the upper parts of the zone.

No doubt the aspen first establishes itself in any given place by

seeds; but the young aspens seen are practically always adventitious shoots which seem gradually to close in on these dry hilltop meadows until at last the ridge is completely covered by them. In these pure stands the shade is dense in summer, so the soil is moister than in the preceding stages. Consequently a new association of herbs replaces those of the dry meadow. *Geranium richardsonii, Castilleja lauta, C. arcuata* (?), *Aquilegia coerulea* and its variety *albiflora, Arabis drummondii, Phacelia sericea, Achillea lanulosa, Microseris* spp., *Fragaria glauca, Senecio atratus, Vicia americana,* and a *Delphinium* are found. In many of the upper aspen groves there is a ground cover consisting of an almost pure stand of *Lathyrus leucanthus. Odostemon repens* and *Pseudocymopterus montanus* are also common in the high aspen groves.

In wide areas on these plateaus are absolutely pure stands of aspen, the trunks often reaching 18 inches in diameter. On some slopes seedlings of Douglas fir, white fir, blue spruce, and (higher up) sub-alpine fir and Engelmann spruce are abundant under the aspens. In these mixed stands the aspens are the largest and dominant trees. This may be due to their more rapid growth, to their greater resistance to fire, or to their general freedom from timbering.

The Douglas fir never stands alone, but it dominates certain rocky slopes. The stages leading to this mixed Douglas fir forest are in general the same as those preceding the aspen, but not so rich in species. Since the Douglas fir succession is more apt to take place on a rocky slope, the pioneers consist of lichens and mosses on the rock faces with crevice plants between them.

The common shrubs are *Odostemon repens, Amelanchier alnifolia, Ribes viscosissimum, Ceanothus fendleri, Sambucus melanocarpa, Artemisia frigida, Symphoricarpos oreophilus, Arctostaphylos uva-ursi,* and *Pachystima myrsinites.* This succession differs from that leading to the aspen in that pioneer trees (*Cercocarpus montanus, C. ledifolius, Picea pungens, Pinus edulis, Juniperus scopulorum*) sometimes precede the climax forest. The blue spruce, which is often thought of as a streamside tree, is common here on dry rocky slopes with the Douglas fir. A forest island of *Picea pungens* in the midst of a dry meadow of cactus, sagebrush, etc., was found on a lava ridge at 9850 feet.

The shade under Douglas firs and other conifers is denser than that formed by the aspens; hence the ground cover is much less luxuriant. Most of the shrubs disappear as the Douglas fir grows up. *Odostemon repens, Juniperus sibirica, Symphoricarpos oreophilus,* and *Rosa pyrifera* endure the shade. *Lathyrus leucanthus, Pseudocymopterus montanus, Poa fendleri, Festuca ovina, Aquilegia flavescens, A. coerulea albiflora, Oxytropis deflexa,* and *Fragaria bracteata* are herbs of the Douglas fir forest. On the whole, the Douglas firs form only medium sized trees in this area. Specimens up to 30 inches in diameter at 4 feet above the ground are sometimes seen.

HYDRARCH SUCCESSION TO CLIMAX MONTANE FOREST.—The hydrarch succession may be found in ponds or in spring swamps which are located where ground water issues from the lava talus above the glacial clay. The soil of these spring banks was the most acidic of any of the samples tested, its pH varying from 6.4 to 6.6. The streamlets are fringed with a lush vegetation consisting of hydrophytic mosses (*Philonotis fontana, Aulacomnium palustre,* etc.) and the herbs *Mimulus guttatus, Aconitum columbianum, Habenaria elegans, H. unalaskensis, Galium boreale, Sidalcea candida, Hypericum formosum, H. scouleri, Polemonium viscosissimum, Thermopsis montana, Carex festiva, Juncus saximontanus, J. balticus, Chamaenerion angustifolium, Veronica alpina, Epilobium ovatifolium, Geranium richardsonii,* and *Mertensia pratensis.* The pioneer shrubs are *Salix subcoerulea, S. bebbiana,* and other willows; they are followed by *Rosa woodsii* and *Lonicera involucrata.* Blue spruce is the pioneer tree in wet soil. Both aspens and Douglas fir seedlings occur on these spring banks, but the former are more common.

There are few aquatics in the deeper lakes such as Fish Lake, for their water is too cold. A gelatinous alga, *Chara, Myriophyllum spicatum,* and species of *Potamogeton* represent the submerged stage. *Batrachium trichophyllum, B. grayanum, Polygonum amphibium,* and *Hippuris vulgaris* surround the central zone of *Potamogeton americanus* in shallow lakes. These amphibious species are usually succeeded by a wet meadow which is often introduced by an almost pure stand of *Eleocharis acicularis. Roripa sinuata* and a small *Ranunculus* are sometimes first. Next on flat shores come *Eleocharis palustris, Juncus balticus, Alopecurus aequalis, Halerpestes cymbalaria,* and

Myosurus aristatus—a small plant usually found on alkali mud at 4000 to 5000 feet in the Great Basin, and seemingly out of place on the shores of mountain lakes at 9700 feet. Next on the moist mud comes a zone of *Rumex mexicanus* with mats of *Taraxia breviflora*. A sedge meadow follows with *Phleum alpinum, Deschampsia caespitosa, Poa ampla, Agropyron tenerum, Stipa columbiana, Hordeum nodosum, Epilobium adenocaulon, Thermopsis montana, Antennaria microphylla, Arnica ocreata,* and species of lupine mixed with the numerous species of *Carex*.

Higher up is a drier meadow of *Dasiophora fruticosa, Pentstemon procerus, Artemisia cana, A. trifida, Achillea lanulosa, Pedicularis parryi, Potentilla pulcherrima* (?), *P. propinqua, Rosa pyrifera,* etc. Where the shores are steep and rocky instead of flat and muddy, narrow amphibious zones may be followed immediately by a fringe of willows or even of aspens.

STREAMSIDE VEGETATION.—In the open, swampy meadows occur along pre-erosion streams. Here hydrophytic sedges, mosses, and rushes are accompanied by clumps of willows, colonies of *Iris missouriensis, Carex nebraskensis, C. lanuginosa, Koeleria cristata, Oryzopsis hymenoides, Sisyrinchium angustifolium, Valeriana edulis,* and *Equisetum hiemale* followed by seedlings of blue spruce and later by aspen. In the higher places *Castilleja exilis, Agropyron tenerum, Aira caespitosa, Potentilla pennsylvanica, Aplopappus uniflora, Antennaria microphylla, Hypericum formosum,* rose, currant, and gooseberry come in.

Where the pre-erosion streams flow through the forest instead of a meadow their vegetation is similar to that of the spring banks. Among the rocks in forest streams grow *Cardamine infausta, C. cordifolia, Mimulus guttatus,* and *Epilobium ovatifolium. Aulacomnium palustre, Philonotis fontana, Conocephalum* sp., and *Marchantia* sp. border the streams. Among them are tufts of *Moneses uniflora, Pyrola chlorantha, Saxifraga rhomboidea, Smilacina stellata*—mostly representatives of zones higher up which are adapted to the cool water of these mountain streams. Wherever there are openings in the forest the willow fringe is seen, usually *Salix subcoerulea* and *S. bebbiana. Alnus tenuifolia* may be with them. Blue spruce seems to be a universal streamside tree of this zone, both in the open and in

the forest. With it are *Filix fragilis, Galium boreale, Phacelia idaho-
ensis, Geranium richardsonii, Aconitum columbianum, Delphinium
subalpinum, Smilacina stellata, Pyrola asarifolia, Veronica wormsk-
joldii, Epilobium adenocaulon, Phleum alpinum, Hypericum for-
mosum, H. scouleri, Castilleja lauta, Polemonium foliosissimum,
Habenaria elegans,* and *H. unalaskensis.*

In the few places where erosion has begun *Equisetum hiemale* is
common on clay or drift banks above the line of present stream
action. On new soils of small floodplains mats of *Halerpestes cym-
balaria* are first; then willows, alders, and blue spruces occupy the
stream deposits. Back farther on drier flats are raspberry, goose-
berry, currant, rose, and sometimes *Juniperus sibirica* with *Poa
canadensis, Fragaria bracteata, Potentilla glaucophylla, Phleum al-
pinum, Mertensia pratensis, Viola nephrophylla,* dandelions, and yar-
row. Aspen comes in on the older floodplain accompanied by the
climax shrubs such as *Symphoricarpos oreophilus, S. longiflorus,
S. racemosus, Odostemon repens,* and the herbs of the aspen stage.

MONTANE CLIMAX FOREST.—The fact that aspen terminates both
xerarch and hydrarch successions might seem to indicate that this
tree is the true climax here. Its edaphic distribution would also class
it as more mesophytic than *Pseudotsuga.* Yet the aspen does not
seem to have a well defined undergrowth. Possibly the inconstant
character of its undergrowth indicates that the aspen is not a true
climax, as has been suggested by some, but a long-time temporary
climax, as others contend. Perhaps it may be merely a stage in a
secondary succession following fire, as some of the rangers think; but
there were few signs of past burns. The aspen may be found succeed-
ing itself, forming dense pure stands. It does so, however, by means
of adventitious shoots, not by seeds germinated in its own shade.
The aspen may be seen also sheltering seedlings of Douglas fir, or it
may come in simultaneously with Douglas fir, or even after it. Both
trees extend up into the Engelmann spruce zone; but the aspen is far
more abundant than is the Douglas fir. The latter extends lower
into the piñon zone than does the aspen, so it must be more tolerant
of dry climate. The aspen is clearly more advanced as to soil re-
quirements. On the other hand, aspen seedlings are more light-toler-
ant; but it colonizes mostly by means of adventitious shoots, and

these do well in shade. Thus this species, like the redwood, is able to maintain itself in conditions of extreme mesophytism although it ranks as a pioneer with respect to its light tolerance. The question whether the aspen or the Douglas fir is the true climax tree of this zone or whether they are ecological equivalents is still an open one.

8. SUBALPINE FOREST ZONE

Overlapping the upper 500–1000 feet of the montane forest, and reaching up to the summits is the subalpine forest. This zone, extending in the main from 9500 to at least 11,000 feet, is characterized by low annual temperatures, a moderate amount of moisture, a short growing season, and high winds. Snow falls as late as June and remains well into July, and the next cold season begins again with snow as early as the last of August.

Without doubt the dominant tree of this association is the Engelmann spruce. It extends from altitudes of 9000 feet at the bases of north facing canyon slopes up to the summits of the plateaus. From 9500 feet up it is the most abundant tree species, and in certain situations the only one. It has unfortunately fallen prey to the ax and the beetle; but tall, straight specimens with trunks 4 feet in diameter are still not uncommon in the dense forest belt just below the lava caps of the plateaus. On the summits and on the rock ridges, where the soil is thin and the wind is strong, it is of course stunted and gnarled; in such places it occurs only in small forest islands.

From 9500 feet up to the base of the lava cap, the Engelmann spruce is accompanied as usual by *Abies lasiocarpa*. The firs here have a narrower range than have the spruces, for neither do they extend as low on the north facing slopes nor do they reach the plateau summits. On south faces their range is from 9800 to about 10,700 feet, the highest altitude noted here for the subalpine fir. The altitudinal limits of this timberline tree seem to be restricted by edaphic rather than by climatic conditions; for wherever the soil is deep, as on the moraines or around alpine lakes, the fir is found. But on the thin, rocky residual lava soils on the summits, the Engelmann spruce stands alone. Throughout the zone the fir is only a subordinate species.

The aspen and the Douglas fir of the montane forest, which are

thoroughly mixed with the spruce and the fir in the lower part of the zone, thin out above. At 10,700 feet the last dwarfed and gnarled aspens were seen just leafing out on June 21 in 1932. Ranger Peterson of Elkhorn Ranger Station reported some limber pines on the summit of Thousand Lake Mt., but I did not find them. At 9800 feet are prostrate specimens of piñon and cedar 1 foot or so in diameter, growing with Douglas fir, aspen, Engelmann spruce, and subalpine fir. TIDESTROM (6) gives 6000 feet as the altitudinal limit of the piñon and RYDBERG (4) locates it at about 8000 feet. Only the yellow pine is lacking at this site to make a complete series.

The subalpine zone is well represented on all three plateaus. Both xerarch and hydrarch successions were found.

XERARCH SUCCESSION TO SUBALPINE CLIMAX.—Xerarch successions are best seen on talus slides and on lava cliffs. On the bare rock are four or five species of crustose lichens: *Acarospora bella*, a black *Acarospora*, *Caloplaca* spp., and *Candelariella vitellina;* semifoliose species such as *Lecanora rubina* and *L. saxicola* follow the xerophytic mosses such as *Tortula muralis* and possibly *Hedwigia*. *Selaginella watsoni* comes in in crevices and on the moss cushions. These early pioneers are followed on rock by the same herbs which introduce the succession on glacial soils and in crevices. On vertical lava walls, *Monardella odoratissima* is nearly always found wherever a little soil has collected. *Polemonium confertum, P. pulcherrimum, Draba aurea, Filix fragilis*, and *Oxyria digyna* are also found in rock crevices in this zone. On deeper soil which is sheltered from the wind, *Mertensia pratensis, Polemonium pulcherrimum, Aquilegia coerulea, Drymocallis fissa, Eriogonum subalpinum, Erigeron flagellaris, Antennaria rosea, Phacelia sericea, Synthyris laciniata, Anemone globosa, Sieversia ciliata, S. turbinata*, and *Potentilla glaucophylla* are common. In moist crevices *Primula parryi* grows luxuriantly. Where grazing is heavy *Achillea lanulosa, Taraxacum officinale, Hymenoxys richardsonii*, and *Dugaldia hoopesii* come in. Shrubs follow the herbs. *Dasiophora fruticosa, Sambucus microbotrys, Ribes lentum, Rubus strigosus*, and *Juniperus sibirica* are most frequent; but *Ribes cereum, Shepherdia canadensis, Lonicera involucrata, Arctostaphylos uva-ursi, Pachystima myrsinites*, and *Odostemon repens* are also found, especially in the lower parts of the zone. In the shelter of boulders on the summits,

Juniperus sibirica and *Ribes lentum* present compact wind-carved thickets in the lee of which the Engelmann spruce starts. The spruce, too, is dwarfed and twisted. One specimen with a trunk 2 feet in diameter was only 18 feet tall. The spruce islands on the summits retain a border of the herbs and shrubs just listed.

Below the rim where the wind is checked the trees become tall and symmetrical. They form continuous forests which cast a shade so dense that the pioneer shrubs and herbs are eliminated. As undergrowth are *Arnica cordifolia*, which may be considered as an indicator of this association, *Saxifraga rhomboidea*, *S. arguta*, *Pyrola secunda*, *Pseudocymopterus montanus*, *Lathyrus leucanthus*, *Thalictrum megacarpum*, *Fragaria bracteata*, *Peltigera* sp., and a *Castilleja* which seemed to be the earliest species through the snow. A species of *Usnea* and *Letharia divaricata* sometimes festoon dead limbs of the spruce. The understory in the climax spruce-fir forest is very sparse, except where there are openings in the forest canopy, for not only is the shade too dense for most plants, but it prevents early thawing of the snow, so that the season is considerably shortened as compared with that of similar situations in the open. Fleshy fungi are frequently abundant on the humus in these forests. Such forms as *Morchella*, *Boletus*, *Clavaria*, and *Lycoperdon* are plentiful.

HYDRARCH SUCCESSION TO SUBALPINE CLIMAX.—Depressions in this zone such as ice-scoured basins, morainic hollows, or troughs dammed up against the lava wall by glacial deposits are occupied by lakes or meadows and surrounded by the subalpine forest. Whether the hydrarch succession attains a forest stage or terminates in the alpine meadow is a question. But whether the alpine meadow is a true climax or merely a long-time temporary climax, it seems to occupy all of the situations which ever have been hydrophytic. In some places the forest is bordered by a belt of Engelmann spruce seedlings which seem to be encroaching upon the meadow surrounding the lake; but usually there is a sharp line between the forest and the meadow, the latter occupying the sediments in the depressions and the former the rocky shores of the basins.

Numerous bodies of water varying from a few feet to a mile or so in diameter are found between 9500 and 10,000 feet. Those at lower altitudes are bordered by the subalpine forest on their north and

east facing shores, but by the montane forest on the south and west faces. In the higher, deeper, and consequently colder lakes there are no submerged or floating aquatics, but near the shores there may be a zone of rushes (*Juncus* spp.) followed by a zone of sedges (*Carex* spp.) in shallow water. In the free water of shallow ponds, however, grow *Hippuris vulgaris, Potamogeton americanus,* and *Polygonum amphibium.* Following the sedge zone on flat shores is a meadow of gentians (*G. heterosepala, G. plebeja,* and others), mosses (*Bryum capillare, Aulacomnium palustre, Climacium americanum, Mnium serratum,* etc.), and *Castilleja* spp., *Equisetum hiemale, Sagina saginoides, Arenaria sajanensis, Taraxia breviflora,* and species of *Antennaria.* One lake at the base of the Thousand Lake lava is surrounded by a slimy white mud on which grows *Leontodon scopulorum,* the dwarf dandelion with heads not more than a quarter of an inch in diameter, on scapes less than 1 inch tall.

On steep shores, which are more common, is a narrow fringe of willows at the water's edge. These are followed closely by subalpine shrubs, *Juniperus sibirica, Ribes roezli, R. niveum,* etc.; then by aspens with an undergrowth of *Fragaria bracteata, Geranium richardsonii,* etc.; and these in turn by the Engelmann spruce and the subalpine fir, which come almost to the water's edge, yet do not seem to occupy any zone which has been a part of the lake itself.

Some ponds are developing a sedge fen with a belt of *Zygadenus elegans* around it.

In the deep valleys, or even in only slight depressions on the tops of the plateaus, there develop alpine meadows which have the appearance of permanent features interrupting the forest and giving the landscape the parklike aspect of the Kaibab Plateau in Arizona.

STREAMSIDE VEGETATION IN SUBALPINE ZONE.—Most of the streams in this zone issue from snow banks or from ponds on the summit and are yet of the pre-erosion type. Increased soil moisture along their courses is the main alteration they make in the subalpine forest. Where they are wide enough they eliminate a band of trees through the forest, thus increasing the illumination at the herb level. Among the rocks in these streams are *Cardamine infausta, C. cordifolia,* and *Primula parryi.* On moist sunny banks is a rich growth of *Aconitum columbianum, Delphinium cockerellii, Mertensia pratensis,*

Allium brevistylum, A. cernuum, Sium cicutaefolium, Aquilegia coeru-
lea, Arnica cordifolia, and the mosses *Amblystegium serpens, Swartzia*
montana, Polytrichum juniperinum, Bryum schleicheri (?), *Brachythe-*
cium idahense, Mnium serratum, Pohlia cruda, and *Pohlia nutans.*
Several species of *Castilleja* and *Lupinus* also occur. *Pentstemon*
whippleanus is found along practically all streams in the subalpine
forest but almost nowhere else.

When the streams flow through open meadows, they often have
a willow fringe, *Salix subcoerulea, S. bebbiana,* and others. The
meadows are in more level places than the forests, so the streams are
more sluggish through them. In the wet mosses are *Viola macloskeyi*
(?), *Gentiana heterosepala, G. plebeja, Parnassia parvifolia, Ranun-*
culus inamoenus, R. calthaeflorus, Moneses uniflora, Pyrola chloran-
tha, Carex engelmannii and other herbs, and *Ribes cereum* and
R. lentum.

No eroding banks nor floodplains were found along the streams in
this zone.

CLIMAX STAGE OF SUBALPINE ZONE.—The montane forest is often
described as the finest of the mountain forests. On these plateaus the
western yellow pine climax, although more open, exceeds it in the
size of the individual trees, and the subalpine forest exceeds it in
both density and size of trees. Where it is protected from high
winds, the Engelmann spruce forest gives the impression of greater
mesophytism even than the aspen groves below it. Since the Aquari-
us is the largest of these plateaus, it is less affected by wind and so
has more extensive areas of these fine forests. In the past 15 years,
nine-tenths of the adult Engelmann spruces on Boulder Mt. have
been destroyed by a bark beetle, *Dendroctonus engelmannii.* The
young spruces are not attacked because they are too resinous. Those
below the rim.seem to have been bothered less than those on the
summit. The moisture conditions may account for this difference,
since more vigorous growth results in greater production of resin.
Climatic cycles may also be a part of the complex, since a series of
dry years would give the beetle the advantage while a wet series
would enable the forest to combat it.

Since the Engelmann spruce is the sole tree species on the top of
the plateau, the next step in the vegetational history is conditioned

by the question, Will the beetle wipe out the forest entirely? It has nearly succeeded on Boulder Mt. at least. Or, will young trees grow up rapidly enough to replace the old ones destroyed? The spruce bears cones while still young and resinous enough to ward off the beetle. Will these young trees remain immune until the beetle is starved out, or will they mature rapidly enough to keep it supplied with food? If the forest is destroyed, what next? Will the entire plateau top develop as a meadow? Or, will another subalpine tree replace the spruce? The subalpine fir is not bothered by this beetle, but it is only the subordinate tree of the subalpine forest here. It has not succeeded so far in establishing itself on the summit. Douglas fir reaches timber line elsewhere, but it, too, is more or less scarce here. The few aspens which have reached the summit are dwarfed and gnarled. Conditions are too hard for them. Elsewhere some forest successors are usually ready to replace the fallen species.

9. Alpine meadows

In the alpine and subalpine zones the depressions usually seem to give rise to a mesophytic meadow which may represent a climax alternating at such altitudes with the spruce forest, or it may represent simply a long-time temporary climax which will eventually give way to the spruce forest. As long as these areas are basins and valleys, they will have a colder and moister climate than the ridges adjoining them. Because of late-lying snows their growing season is shorter. Because of protection from wind their evaporation is less. Because of higher water-table their soil moisture is greater. And because of cold-air drainage into them their temperatures are lower. In short, their climate is not that of the neighboring forest. If every climate has its climax, we should not expect the same climax in these two situations.

Some of the same species found in these mountain meadows are typical also of snow bank vegetation. Around snow banks on slopes we get much the same climatic conditions—short season, low temperature, and high moisture. Plants found in such places must be tolerant of such conditions, although they may be unable to compete elsewhere with other species. Probably not until the depressions are filled so that their climate approaches that on the slopes

surrounding them will conditions allow the forest to replace the meadow.

The depressions on the summit have thinner soil and are shallow and therefore windier than those in the zone below. The dry alpine scrub association eventually supplants the wet meadow species in these places. Below the subalpine zone where the season is longer, the forest may replace the meadow. In both of these situations the mesophytic alpine meadow association is but a subclimax.

In this plateau region the typical meadow formation occurs in valleys and other depressions from 9000 to 11,000 feet and around snow banks on the summits. Small pre-erosion streams make their way through many of them. The alpine meadow here is apparently always reached through a hydrarch succession, which is one of the arguments for considering it but a temporary climax.

Deep water at such altitudes is too cold for vegetation, but in shallow water, rushes (*Eleocharis acicularis, Juncus filiformis, J. saximontana*), sedges (*Carex ebenea, C. siccata, C. variabilis,* and others), and aquatic mosses (*Amblystegium riparium* vars. *fluitans* and *longifolium, Crataneuron filicinum, Bryum caespiticium, Drepanocladus aduncus intermedius, D. uncinatus, Mnium affine rugicum,* and *Philonotis fontana*) appear as the hydrophytic pioneers. Some meadows are occupied by an almost solid stand of this association. Such a spongy turf seems to develop most frequently in silty meadows in this zone. Streams running through the sedge fen are bordered by *Salix pseudomyrsinites aequalis* (?), *S. subcoerulea,* and other willows.

Rocky floors of valleys do not seem to be favorable for the formation of a sedge turf. Instead they usually develop the typical mountain meadow association. Summit Valley at 9500 feet is a good example. Here the pioneer aquatic rushes and sedges are followed first by *Caltha rotundifolia,* which often pushes its flower stalks through the snow. Soon after it comes *Polygonum bistortoides* and *Pedicularis groenlandica* or its variety *surrecta.* These three species and *Phleum alpinum* are always to be found in the mountain meadows of this region. *Deschampsia caespitosa, Poa longiligula, Gentiana heterosepala, G. plebeja, G. calycosa,* and *G. affinis* may also be found in the wet meadow zone. Next comes in a somewhat drier belt of such

forms as *Zygadenus elegans, Ranunculus calthaeflorus, Clementsia rhodantha, Dodecatheon pauciflorum, Veronica wormskjoldii, Saxifraga rhomboidea, Sieversia brevifolia* (?), *S. ciliata, Trifolium rydbergii, Poa sandbergii, Thermopsis montana, Potentilla effusum, P. concinna, P. monspeliensis, Lupinus parviflorus, Antennaria aprica, A. parvifolia, Senecio petrophilus,* and several species of *Castilleja* (*C. lauta, C. arcuata,* and others). This stage probably represents the climax of this series. In badly overgrazed meadows the climax forms may be partly or wholly replaced by such weedy species as *Hymenoxys richardsonii, Antennaria aprica, Androsace diffusa, Orthocarpa lutea,* yarrow, and dandelion. Along the streams are *Delphinium subalpinum, Aconitum columbianum, Mimulus guttatus, Cardamine infausta,* and sometimes willows.

There is usually a transition belt separating this meadow from the spruce forest. It is dominated by *Artemisia cana,* which is accompanied by *Dasiophora fruticosa, Agrostis hiemalis, Dugaldia hoopesii,* and *Eriogonum subalpinum.* The mesophytic meadows on the tops of the plateaus which adjoin the dry alpine scrub instead of the spruce forest lack the *Artemisia cana* border, which would seem to indicate that this sagebrush association is an outpost from the forest formation, rather than a successor to the meadow.

Certain alpine species which are uncommon below 10,000 feet are found in the higher rocky meadows. *Oxyria digyna, Sibbaldia procumbens, Primula parryi, Synthyris laciniata, Polemonium confertum, Filix fragilis,* and *Allium cernuum* are all found in moist crevices on high shaded cliffs.

10. ALPINE SCRUB CLIMAX

On the summits of the High Plateaus, the subalpine forests often alternate with the dry alpine climax which occupies places where the soil is thin and exposure to the wind is great. The reduced size of the plants is evidence of the extreme exposure and the short season. This association seems to terminate a xerarch succession, but there is some evidence that a wet meadow may be replaced by it.

Placodium cinnabarinum and other crustose lichens (*Acarospora, Caloplaca, Lecanora rubina, L. saxicola,* and *Candelariella vitellina*) gain foothold on the smaller rock fragments as well as on the exposed

bedrock. Some foliose forms such as *Parmelia conspersa* and *Gyrophora hyperborea* also occur. The mosses *Tortula muralis* and *Hypnum revolutum* are seen in crevices with *Selaginella watsoni*. Apparently loose on stony soil is *Parmelia molliuscula*, a pale green foliose form which is blown about by the wind. Typical xerophytic alpine herbs follow. These are characterized usually by relatively large underground structures topped by a rosette of minute, often fleshy or hairy leaves and a short slender scape bearing the relatively large flowers. They develop rapidly when once the snow is gone, and mature their fruits before the short growing season is over. The commonest of these on the plateau summits are *Oreobroma pygmaea*, a fleshy leaved relative of our eastern spring beauty, *Androsace subumbellata*, *A. carinata*, *Sedum stenopetalum*, *Polygonum watsonii*, *Thlaspi glaucum* (?), *T. coloradense* (?), and a new *Thlaspi*, *Erigeron compositus* and its varieties *incertus* and *trifidus*, *Arenaria sajanensis*, *Draba mongollonica*, *D. crassifolia*, *Cerastium beeringianum*, *Artemisia scopulorum*, *Achillea lanulosa alpicola*, *Senecio petrophilus*, and species of *Antennaria*. As the soil deepens the alpine grasses take possession. The dominant species is *Festuca ovina* and its variety *brachyphyllum*. *F. rubra*, *F. thurberi*, *Agropyron tenerum*, and a little black sedge are also common at this stage. Between the tufts of grasses *Pseudocymopterus montanus*, *Achillea*, and *Sibbaldia procumbens* are found. The short fescue, *Festuca ovina*, gives the tone to these summit landscapes, but counts made in a number of meter quadrats show that other species, such as *Oreobroma pygmaea*, *Androsace subumbellata*, *Achillea*, and *Pseudocymopterus montanus*, although minute and inconspicuous, are very numerous.

The shallow troughs which drain the ponds and lakes on the summits may begin with a wet meadow association. Along the water courses are sedges, but on the wet banks grows *Caltha rotundifolia* in fairly pure stands. *Polygonum bistortoides* (and sometimes its variety *linearifolia*) and *Pedicularis groenlandica* are next, then *Phleum alpinum*, *Deschampsia caespitosa*, *Stipa columbiana*, *Saxifraga rhomboidea*, a white and a new purple flowered *Thlaspi*, *Sieversia turbinata*, *Phacelia sericea*, *Polemonium confertum*, *Potentilla concinna*, *P. glaucophylla*, *Sibbaldia procumbens*, *Senecio petrophilus*, and *Pseudocymopterus montanus* come in on the mid-slopes; at the top of

the slopes are the grasses and their associates. Here the wet meadow seems to be a pioneer stage preceding the dry tundra.

Wherever lava blocks afford some protection from the wind, or on leeward slopes, taller, more mesophytic herbs come in. These include blue and yellow columbines, *Anemone globosa*, *Phacelia sericea*, *Carex festiva*, *Mertensia pratensis*, *Sieversia triflora*, *S. turbinata*, *S. ciliata*, *Potentilla diversifolia*, *Drymocallis fissa*, *Heuchera parvifolia*, *Polemonium confertum*, a white *Polemonium*, *Erigeron* spp., and taller grasses such as *Poa sandbergii*, *Blepharineuron tricholepis*, *Koeleria cristata*, and *Deschampsia caespitosa*.

These rocks and taller herbs sometimes afford enough protection to permit the alpine shrubs *Juniperus sibirica* and *Ribes lentum* to come in. These shrub mats are more common on the central part of Fish Lake Plateau where large lava blocks were scattered by the glacier than on the other plateaus where the rock fragments are mostly small. They probably introduce the subalpine forest islands which dot the landscape on the Aquarius Plateau, but on the smaller plateaus the forests so developed are seldom much more than mere clumps of stunted and gnarled timberline trees. Whether they can compete against the wind and eventually establish a real climax spruce forest is a question. The timberline here seems to be determined by wind and by depth of soil rather than by temperature. At present the summits of all these High Plateaus represent a line of tension between the subalpine forest climax and the alpine scrub climax. Whether the true climax here is the subalpine forest, which is often long delayed owing to the slow disintegration of the rock and to desiccation by the wind, or the xerophytic alpine scrub which occasionally may be preceded by a more mesophytic subclimax which will eventually give way to it, is yet unsettled. Possibly both associations are true climax formations here, but each in its own climatic zone; for the climate on a broad plateau like the Aquarius Plateau may differ considerably from that on an isolated peak at the same altitude. At any rate, on the broader plateaus such as Aquarius Plateau, the two associations run rather parallel while on small tables like Fish Lake Plateau and Thousand Lake Mt. and on peaks like Mt. Marvine, where the wind is stronger, the alpine scrub takes the lead.

Plant list

I, southern desert zone; IA, northern desert zone; IB, alkali desert
II, southern semidesert zone; IIA, northern semidesert zone
III, basal (western yellow pine) zone
IV, montane forest zone
V, subalpine forest zone
VI, alpine meadow climax
VII, alpine scrub climax

X, xerophytic; XX, extremely xerophytic
XM, xero-mesophytic
M, mesophytic
HM, hydro-mesophytic
H, hydrophytic
R, ruderal

I. Lichens

(identified by the late Dr. Plitt of Baltimore, Md.)

Acarospora bella (Nyl.) Jatta. . . . . .crustose on lava
A. spp. . . . . .crustose on lava
Caloplaca spp. . . . . .crustose on lava
Candelariella vitellina Mull. Ary. . . . . .crustose on lava
Gyrophora hyperborea (Hoffm.) Mudd. . . . . .foliose on rocky soil
Lecanora rubina (Vill.) Wain. . . . . .semifoliose on rock
L. saxicola (Poll.) Ach. . . . . .semifoliose on rock
L. spp. (aspicilia?). . . . . .semifoliose on rock
L. divaricata (L.) Ach. . . . . .fruticose on spruces
Parmelia conspersa Ach. . . . . .foliose on rock, soil
P. molliuscula Ach. . . . . .foliose on rock
Peltigera spp. . . . . .foliose on moist soil
Usnea spp. . . . . .fruticose on spruces

II. Mosses

(identified by Dr. Seville Flowers)

Amblystegium riparium var. fluitans L. & S. . . . . VI HM
A. riparium var. longifolium (Schultz) B. & S. . . . . . . VI HM
A. serpens B. & S. . . . . V HM
Aulacomnium palustre Schwaegr. . . . . IV, VI HM
Brachythecium idahense R. & C. V M

Bryum caespiticium L........................... VI HM
B. capillare L................................... VI HM
B. schleicheri Schwaegr. (?)....................... V M
B. spp... VI HM
Climacium americanum Brid..................... VI HM
Cratoneuron filicinum (L.) Roth.................. VI HM
Drepanocladus aduncus var. Kneiffii form intermedi-
    us (B. & S.) Moenken....................... VI HM
D. uncinatus (Hedw.) Warnst.................... VI HM
Hypnum revolutum Nutt........................ VII X
Mnium affine var. rugicum B. & S............... VI HM
M. serratum Schrad............................. V M
Philonotis fontana (L.) Brid.............. IV, V, VI HM
Pohlia cruda (L.) Lindb........................ VI HM
P. nutans (Schreb.) Lindb....................... V M
Polytrichum juniperinum Willd................... V M
Swartzia montana (Lank.) Lindb. .... V HM
Tortula muralis (L.) Hedw..................... VII XX

III. LIVERWORTS

Conocephalum spp...................... III, IV, V HM
Marchantia spp........................ III, IV, V HM

IV. VASCULAR PLANTS

A. Trees

Abies concolor Lindl............................ IV M
A. lasiocarpa (Hook.) Nutt...................... V M
Acer interius Britton......................... I, II HM
Alnus tenuifolia Nutt.......................... IV HM
Amelanchier alnifolia Nutt.................. III, IV XM
A. oreophila A. Nels.......................... IIA XM
A. utahensis Koehne........................... I X
Betula fontinalis Sarg.......................II–IV HM
Celtis rugulosa Rydb........................... I HM
Cercocarpus intricatus Wats.................... II X
C. ledifolius Nutt.................. .... IIA, III, IV XM
C. parvifolius Nutt..................... IIA, III, IV XM

Fraxinus anomala Torr........................... I HM
Juniperus scopulorum Sarg.................. II, III XM
J. utahensis (Engelm.) Lemm.................... II XM
Picea engelmannii (Parry) Engelm................ V M
P. pungens Engelm................. II, III, IV HM, XM
Pinus aristata Engelm......................... III XM
P. edulis Engelm................................ II XM
P. flexilis James................................ V XM
P. ponderosa Dougl............................. III XM
Populus angustifolia James...................... II HM
P. aurea Tidest................................ IV M
P. fremontii S. Watson.......................... I HM
P. trichocarpa T. & G.......................... II HM
Prunus melanocarpa (A. Nels.) Rydb........ II, III, IV M
Pseudotsuga mucronata (Raf.) Sudw........ IV, III, V M, XM
Quercus utahensis Rydb.................... IIA, III XM
Salix lasiandra Nutt............................ I HM

B. Shrubs

Arctostaphylos platyphylla (A. Gray) Kuntze...... III XM
A. uva-ursi (L.) Spreng................... III, IV, V XM
Artemisia cana Pursh....................... V, VI M
A. filifolia Torr................................. I X
A. frigida Willd............................. III, IV X
A. tridentata Nutt...................... IA to IV X
A. tripartita Rydb. (?)...................... V, VI HM
Atriplex canescens (Pursh) Nutt................. IB X
A. confertifolia (Torr. & Gray) S. Watson.......... IB X
A. spp... IB X
Ceanothus fendleri Gray.................... III, IV X
C. velutinus Dougl.............................. IV XM
Chrysothamnus graveolens (Nutt.) Greene.... IIA to IV X
Clematis ligusticifolia Nutt...................... I HM
Coleosanthus microphylla Gray.................... I X
Cornus stolonifera Michx.................... II, III HM
Cowania stansburiana Torr.................. I, II, III X
Dasiophora fruticosa (L.) Rydb................. VI M

Ephedra torreyana S. Watson.................... I X
E. viridis Coville............................... I X
Eriogonum corymbosum Benth................... I X
E. effusum Nutt............................... IIA XM
E. microthecum Nutt. (?)..................... IIA XM
E. (near polifolium Benth.)..................... IIA XM
Fallugia paradoxa (Don) Endl............... I, II, III X
Holodiscus dumosus (Nutt.) Heller.............. IIA XM
Juniperus sibirica Burgsd............. III, IV, V, VII XM
Lepargyrea argentea (Pursh) Greene............... IV M
L. canadensis (L.) Greene.................... IV, V M
L. rotundifolia (Parry) Greene................ I to III X
Lonicera involucrata Banks..................... IV M
Odostemon fremontii (Torr.) Rydb................. I XM
O. repens (Lindl.) Cockerell............... III, IV, V M
Opulaster malvaceus (Greene) Kuntze........... IV, V M
Pachystima myrsinites (Pursh) Raf........ III, IV, V M
Philadelphus microphyllus Gray................... I X
Phoradendron juniperinum Engelm.... (parasite on juniper)
Purshia tridentata DC...................... III, IV XM
Rhus trilobata Nutt. (?)........................ I HM
R. utahensis L. N. Goodding..................... I X
Ribes cereum Dougl.......................... IV, V XM
R. inebrians Lindl.......................... III, IV XM
R. lentum (Jones) Coville & Rose... ....V, VII XM
R. leptanthum Gray............................. III XM
R. niveum Lindl................................ V M
R. roezli Regel................................ V M
R. viscosissimum Pursh (?).................. IV, V M
Rosa fendleri Crépin (?)..................... III, IV XM
R. manca Greene (?)............................ IV M
R. puberulenta Rydb........................... I HM
R. pyrifera Rydb. (?).......................... IV M
R. woodsii Lindl.............................. V HM
Rubus hispidus L.............................. IV M
R. strigosus Michx..................... IV, V, VII XM
Salix bebbiana Sarg........................... V HM

S. exigua Nutt................................... IV HM
S. lutea Nutt. (?)................................ IV HM
S. pseudomyrsinites var. aequalis Anderrs. (?)....... V HM
S. subcoerulea Piper............................. IV HM
Sambucus melanocarpa Gray................. III, IV XM
S. microbotrys Rydb......................... IV, V M
Sarcobatus vermiculatus (Hook.) Torr.............. IC X
Sericotheca microphylla Rydb................... IIA XM
Symphoricarpos longiflorus Gray................. IV X
S. oreophilus Gray............................ IV XM to M
S. racemosus Michx......................... III, IV XM
Tetradymia canescens DC. var. inermis Gray
                                    IIA, III, IV X
Toxicodendron rydbergii Small..................... I XM
Yucca harrimaniae Trel........................ I, II X
Y. spp... I, II X

C. Herbs

Abronia elliptica A. Nels...................... I, II X
A. spp.. III XM
Achillea lanulosa var. alpicola Rydb............. V, VI M
A. millefolium L.......................... III to V R
Aconitum columbianum Nutt................... IV, V HM
Agastache urticifolia (Benth.) Rydb............... V M
Agoseris purpurea (Gray) Greene................ IV XM
Agropyron spicatum (Pursh) Scribn. & Wheeler..... III XM
A. tenerum Vasey............................. IV HM
Agrostis hiemalis (Wolf) B.S.P................. III, IV HM
Allionia spp................................... I X
Allium brandegii Hook...................... III, IV HM
A. brevistylum Wats........................... V HM
A. cernuum Roth............................. VII HM
Allocarya scopulorum Greene.................... IV M
Alopecurus aequalis Sobol..................... IV HM
A. geniculatus L.............................. IV HM
Ambrosia psilostachya DC...................... I X
Andropogon scoparius Michx.................... I X

Androsace carinata Torr. (?)................... VII X
A. diffusa Small............................ IV, V M
A. subumbellata (A. Nels.) Small.............. VII X
Anemone globosa Nutt........................ VI HM
Antennaria aprica Greene..................... III X
A. dimorpha (Nutt.) Torr. & Gray............. VI M
A. microphylla Rydb......................... IV HM
A. parvifolium Nutt......................... VI HM
Apocynum androsaemifolium L................. IV M
Aplopappus spp.............................. I X
Aquilegia coerulea James.................. IV, V, VI M
A. coerulea albiflora Gray................. IV, V, VI M
A. flavescens S. Wats....................... VII XM
A. pinetorum Tidest. (?).................... IV M
Arabis drummondii Gray...................... IV M
Arenaria aculeata Wats....................... I X
A. sajanensis Willd......................... VII X
Argemone hispida Gray....................... I R
Aristida fendleriana Steud.................. IV X
Arnica cordifolia Hook...................... V M
A. ocreata A. Nels.......................... V HM
Artemisia scopulorum Gray................... VII X
A. wrightii Gray........................... II X
Asclepias cryptoceras Wats.................. I XM
A. incarnata L.............................. I XM
A. labriformis M. E. Jones................. I X
Aster adscandens Lindl. (?)................. IV HM
A. glaucodes Blake.......................... IV HM
Astragalus diversifolius Gray............... II XM
A. thompsonae S. Wats.................... I, II X
Atriplex spp............................... IB X
Batrachium grayanum (Freyn.) Rydb.......... IV H
B. trichophyllum (Chaix.) Barsch.......... IV, V H
Blepharineuron tricholepis (Torr.) Nash.... VII X
Bouteloua gracilis (H.B.K.) Lag........... I to III X
B. hirsuta Lag............................. IV X
Bromus ciliatus L.......................... IV HM

B. inermis Leyss.............................. III R
Calochortus nuttallii T. & G.................. II, III XM
Caltha rotundifolia Greene...................... VI HM
Campanula parryi Gray........................ IV HM
C. uniflora L. (?)............................. VII X
Cardamine cordifolia Gray..................... V HM
C. infausta Greene...................... III to VI HM
Carex ebenea Rydb............................. VI HM
C. engelmannii Bailey................ .... VI HM
C. festiva Dewey............................. VII X
C. lanuginosa Michx.......................... IV HM
C. multinoda Bailey........................... IV HM
C. siccata Dewey................... .... V, VI HM
C. variabilis Bailey........................... VI HM
C. spp...................................... . VI HM
Castilleja arcuata Rydb..................... ... V M
C. exilis A. Nels............................. IV HM
C. flava A. Nels........................... . IV X
C. lauta A. Nels.............................. V M
C. linariaefolia Benth........... .... V M
C. linoides Gray (?)......................... IIA X
C. miniata Dougl. (?)........................ V M
C. parvula Rydb. (?)......................... IV X
C. rhexifolia Rydb. (?)................... IV, V HM
Catabrosa aquatica (L.) Beauv................ IV, V H
Cenchrus pauciflorus Benth...................... I HM
Cerastium beeringianum C. & S.................. VII X
Chaenactis alpina (Gray) Jones (?).............. VII X
C. douglasii H. & A................... IIA, III, IV X
Chamaesyce fendleri (T. & G.) Small............ I, II X
Chamaenerion angustifolium (L.) Scop............ IV HM
Chrysopsis spp................................. I HM
Chrysothamnus spp......................... III, IV X
Chylismia spp................................. I X
Clematis pseudoalpina (Kuntze) A. Nels........... III XM
Clementsia rhodantha (Gray) Rose............... VI HM
Collomia linearis Nutt........................ IV M

Comandra pallida A. DC.............. IIA, III, IV XM
Corallorrhiza maculata Raf. parasite on roots....... III XM
Crepis acuminata Nutt......................... IV M
Datura meteloides DC........................... I XM
Delphinium cockerellii A. Nels................... V M
D. subalpinum (Gray) A. Nels................ IV, V HM
Deschampsia caespitosa (L.) Beauv.............. VI HM
Dodecatheon pauciflorum (Durand) Greene........ VI HM
Draba aurea Vahl.............................. V X
D. crassifolia Graham (?)...................... VII M
D. mongollonica Greene........................ VII X
D. spectabilis Greene (?)...................... VII X
Drymocallis fissa (Nutt.) Rydb.................. VI M
D. spp.. VI M
Dugaldia hoopesii (Gray) Rydb................. VI M
Echinocactus xeranthemoides Coulter............. I X
E. spp.. I X
Echinocereus fendleri (Engelm.) Rumpl. (?).... ..... I X
E. goniocanthus (Engelm.) Lem. (?)........... ... I X
E. mojavensis (Engelm.) Rumpl................. I X
E. triglochidiatus (in Britton & Rose).............. I X
Eleocharis acicularis (L.) Roem. & Schultz....... IV, V H
E. palustris (L.) Roem. & Schultz.............. IV, V H
Epilobium adenocaulon Hausskn................ IV, V HM
E. hornemannii Reichert (?).................. V, VII HM
E. ovatifolium Rydb............................ IV H
Equisetum arvense L...................... III, IV, V HM
E. hiemale L................................ III, IV H
Erigeron argentatus Gray...................... I, II X
E. bloomeri Gray.............................. IV X
E. compositus Pursh......................... V, VII X
E. compositus discoideus Gray or incertus A. Nels... VII X
E. compositus trifidus Gray.................... VII X
E. eatonii Gray (?)............................ IV M
E. flagellaris Gray............................ IV X
E. formosissimum Greene...................... VI M
E. leiomerus Gray............................ I, II X

E. macranthus Nutt............................ VI M
E. salsuginosus Gray.......................... IV HM
E. superbus Greene............................ VI HM
E. ursinus D. C. Eaton (?)................... IV, V M
E. vetensis Rydb.............................. III XM
Eriogonum alatum Torr........................ III XM
E. inflatum Torr................................ I X
E. jamesii Benth................................ IV X
E. ovalifolium Nutt.... .... I, II X
E. piperi Greene............................... VI M
E. racemosum Nutt..................... IIA, III, IV X
E. stellatum Benth......................... III, IV X
E. subalpinum Greene......................... VI M
E. umbellatum Torr......................... III, IV XM
E. spp............................... other species in all zones
Euphorbia lurida Engelm. or
    Tithymalus luridus (Engelm.) Woot. & Standl..... III XM
Eurotia lanata (Pursh) Moq...................... IB X
Festuca ovina L............................. V, VII X, XM
F. ovina brachyphylla Schult.................. V, VII X
F. idahoensis Elmer............................ IV X
F. thurberi Vasey........................... V, VII XM
Filix fragilis (L.) Underw................. IV to VII XM
Fragaria bracteata Heller....................... V HM
F. glauca (S. Wats.) Rydb....................... V HM
Franseria acanthicarpa (Hook.) Coville............. I X
Frasera speciosa Griseb........................ IV, V HM
Galium boreale L............................... IV HM
Gayophytum ramosissimum T. & G................. IV X
Gentiana affinis Griseb......................... V HM
G. calycosa Griseb. (?)......................... VI HM
G. elegans A. Nels.............................. IV HM
G. heterosepala Engelm......................... VI HM
G. plebeja Cham............................... VI HM
Geranium carolinianum L....................... IV XM
G. richardsonii F. & M...................... IV, V M
G. viscosissimum Fisch. & Mey............. IV to VII M

Geum macrophyllum Willd...................... IV HM
G. strictum Ait............................... IV HM
Gilia aggregata (Pursh) Spreng............... III, IV X
G. dixonae A. Nels. 1934....................... I X
G. pulchella Dougl............................ IV X
Glyceria striata Hitch........................ IV HM
Gnaphalium uliginosum L....................... VI HM
Gutierrezia filifolia Greene.................. III X
G. spp....................................... III, IV X
Habenaria elegans (Lindl.) Boland............. IV HM
H. unalaskensis (Spreng.) Wats................ IV HM
Halerpestes cymbalaria (Pursh) Greene........ I to V HM
Heuchera parvifolia Nutt...................... V M
Hilaria jamesii (Torr.) Benth................. I, II X
Hippuris vulgaris L........................... IV, V H
Hordeum nodosum L............................ IV HM
Hymenoxys helenioides Rydb.................... V, VI HM
H. odorata (DC.) Britt........................ III X
H. richardsonii (Hook.) Ckll.................. V R
Hypericum formosum H.B.K...................... IV HM
H. scouleri Hook.............................. IV HM
Iris missouriensis Nutt....................... IV HM
Iva axillaris Pursh........................... I X
Jonesiella asclepiadoides Rydb................ I X
Juncus balticus Willd......................... III to VI H
J. filiformis L.............................. IV, V HM
J. saximontanus A. Nels...................... IV, V HM
Kelloggia galioides Torr...................... IV M
Koeleria cristata L........................... VI HM
Lappula floribunda (Lehm.) Greene............. IV XM
L. occidentalis (S. Wats.) Greene............. IV XM
Lathyrus leucanthus Rydb..................... IV, V M
L. spp....................................... V M
Lavauxia flava A. Nels...................... IV, V M
L. howardii M. E. Jones...................... V HM
Leontodon scopulorum Rydb.................... V HM
Lepidium jonesii Rydb......................... I XM

Lesquerella alpina (Nutt.) Wats................... III X
Ligusticum porteri Coulter & Rose................ IV M
Linum lewisii Pursh............................. IV M
Lithospermum multiflorum Torr. Wats............. IV M
Lotus wrightii (Gray) Greene................. III, IV XM
Lupinus alpestris A. Nels.................. III, IV, V XM
L. argenteus Nutt............................. IIA X
L. lepidus Dougl................................ III XM
L. parviflorus Nutt.............................. V HM
L. pseudoparviflorus Rydb. (?).................... V HM
L. spp.................................. II to VII
Lygodesmia exigua Gray........................ IIA X
L. juncea (Pursh) D. Don......................... II X
L. spinosa Nutt. or Pleiacanthus spinosus (Nutt.) Rydb.
                                        II to IV X
Melilotus alba Desv............................. I R, HM
M. officinalis (L.) Lam......................... I R, HM
Mertensia arizonica Greene (?).................... IV HM
M. pratensis Heller......................... IV, V HM
Microseris spp.................................. IV X
Mimulus guttatus DC......................... IV, V H
Mitella spp..................................... V M
Monardella odoratissima Greene.................. V XM
Moneses uniflora (L.) Gray....................... V HM
Montia chamissoi (Ledeb.) Tidest................. V HM
Muhlenbergia asperifolia Nees. & Mey............ IB HM
Myosurus aristatus Benth.................... IV, V HM
Myriophyllum spicatum L......................... IV H
Opuntia fragilis (Nutt.) Haw.............. II, III, IV X
O. polyacantha Haw. (?)...................... I, II X
O. rhodantha Schum.............................. II X
O. rutila Nutt.............................. I, II X
O. xanthostemma Schum.......................... II X
O. spp..................................... I, II X
Oreobroma pygmaeum (Gray) Howell.............. VII X
Oreocarya flava A. Nels. (?)..................... I X
O. setosissima (Gray) Greene (?)................. IV XM

O. (near suffruticosa (Torr.) Greene)............. III X
Oreoxis alpina (Gray) C. & R..................... V M
Orobanche ludoviciana Nutt. parasitic on Chrysotham-
    nus... II X
Orthocarpus luteus Nutt......................... V M
Oryzopsis hymenoides (Roem. & Schult.) Ricker..... II X
Osmorrhiza obtusa (Coulter & Rose) Fernald..... IV, V M
Oxyria digyna (L.) Hill......................... VII XM
Oxytropis deflexa (Pall.) DC. (?)................. V XM
Pachylophus macroglottis Rydb................... IV X
Parnassia parvifolia DC......................... V HM
Pedicularis bracteosa Benth..................... VI XM
P. groenlandica Retz............................ VI HM
P. groenlandica surrecta (Benth.) Greene.......... VI HM
P. parryi Gray................................. VI M
Pediocactus simpsonii (Engelm.) Britton & Rose. III, IV X
Pentstemon arenicola A. Nels.................... III X
P. caespitosus Nutt............................. II XM
P. eatonii undosus M.E. Jones................... II X
P. ophianthus Pennell........................... II XM
P. pachyphyllus Gray........................... III XM
P. procerus Dougl.............................. VI M
P. procerus pulverens Pennell................... . VI M
P. strictus Benth............................... IV X
P. subglaber Rydb.............................. V M
P. torreyi Gray (?)............................. III XM
P. whippleanus Gray............................ V HM
P. spp....................................... I to VII X to M
Phacelia corrugata A. Nels...................... I XM
P. idahoensis Henders.......................... IV M
P. sericea Gray....................... IV, V, VI, VII M, XM
P. spp.. IIA X
Phleum alpinum L.............................. VI HM
Phlox austromontana Coville.................... III XM
P. canescens Torr. & Gray...................... I XM
P. cortezana A. Nels........................... V M
P. stansburyi (Torr.) Heller.................. III, IV XM

Physaria didymocarpa Gray..................... III XM
Poa ampla Merr................................. VI M
P. fendleriana (Steud.) Vasey................. IV, V M
P. longiligula Scribn. & Wms..................... V M
P. pratensis L.............................. IV, V M
P. sandbergii Vasey........................ VI, VII M
Polemonium albiflorum Eastw. (?)............... VII X
P. confertum Gray............................. VII X
P. foliosissimum Gray (?)...................... IV HM
P. pulcherrimum Hook.......................... VII X
P. viscosum Nutt. (?).......................... VII X
Polygonum amphibium L..................... IV, V H
P. bistortoides Pursh..................... VI, VII HM
P. bistortoides linearifolia S. Wats........... VI, VII HM
P. watsonii Small.............................. VII X
Potamogeton americanus Schlecht. & Cham...... IV, V H
P. spp.................................... IV, V H
Potentilla concinna Rich...................... VII X
P. (near concinnaeformis Rydb.)................ VII X
P. dichroa Rydb............................... III XM
P. diversifolia Lehm.......................... IV XM
P. effusa Dougl., Lehm........................ V HM
P. fastigiata Nutt............................ IV HM
P. glaucophylla Lehm........... IV, HM to VII XM
P. gracilis Dougl............................. IV M
P. hippiana Lehm. (?)......................... IV HM
P. intermittens Rydb. (?)..................... IV HM
P. monspeliensis L............................ V HM
P. nuttallii Lehm............................. IV HM
P. pennsylvanica L............................ III HM
P. propinqua Rydb............................. IV HM
P. spp..................................... IV–VII
Primula parryi Gray........................ V, VII HM
Pseudocymopterus montanus (Gray) Coulter & Rose
                                        III, IV, V XM
P. spp... VII X
Pyrola asarifolia Michx........ .... V HM

P. chlorantha Sw............................... V HM
P. secunda L............................... III, IV HM
Quamoclidion froebelii (Behr.) Standl............... I XM
Ranunculus alpeophilus A. Nels................. V HM
R. calthaeflorus Greene....................... VI HM
R. cardiophyllus Hook........................ VI HM
R. cymbalaria Pursh or Halerpestes cymbalaria
    (Pursh) Greene........................... I to V HM
R. escholtzii Schlect........................... IV HM
R. inamoenus Greene......................... V HM
R. montanensis Rydb......................... IV HM
R. sceleratus L............................... III HM
Roripa curvipes Greene........................ V HM
R. sinuata (Nutt.) A.S.H...................... IV HM
Rumex mexicanus Meisn....................... IV HM
Sagina saginoides (L.) Britton.................. VII HM
Salsola pestifer A. Nels........................ I X
Saxifraga arguta D. Don....................... V M
S. integrifolia sierrae Coville.................. V M
S. rhomboidea Greene......................... V HM
Sedum stenopetalum Pursh.................... VII X
S. spp....................................... VII X
Selaginella mutica D. C. Eaton.................. II X
S. watsoni Underw........................... VII X
Senecio atratus Greene....   .... V M
S. crocatus Rydb............................. IV HM
S. cymbalarioides Nutt........................ VI M
S. multilobatus Torr. & Gray.................. IV X
S. mutabilis Greene........................... IV HM
S. petrophilus Greene......................... VII X
S. purshiana Nutt. (?)........................ VII X
S. triangularis Hook.......................... V X
S. spp........... .....several other species in zones III–VII
Sibbaldia procumbens L....................... VII XM
Sidalcea candida Gray........................ IV HM
Sieversia ciliata G. Don...................... VI, VII M
S. triflora (Pursh) R. Br...... .... VI, VII XM

S. turbinata (Rydb.) Greene.................... VII X

Silene antirrhinus L............................... V M

Sisyrinchium angustifolium Nutt................... IV HM

Sitanion hystrix (Nutt.) J. C. Smith.............. III X

Sium cicutaefolium Gmelin....................... V HM

Smilacina stellata (L.) Desf...................... III HM

Solidago bigelovii Gray.......................... IIA X

Sophia filipes (Gray) Heller..................... VII X

S. hartwegiana (Fourn.) Greene.................. IV HM

Sphaeralcea grossulariaefolia (H. & A.) Rydb...... I, II X

Stanleya integrifolia James....................... I HM

Stipa columbiana Macoun................ IV to VII X

S. comata intermedia Scribn..................... IV X

S. speciosa Trin. & Rupr........................ I, II X

Synthyris laciniata (Gray) Rydb.................. V HM

Taraxacum officinale Web.................. II to VII R

Taraxia breviflora (T. & G.) Nutt.............. IV, V HM

T. subacaulis (Pursh) Rydb. (?)................... V M

Thalesia fasciculata (Nutt.) Britton, parasite on Arte-
misia...................................... IIA X

Thalictrum fendleri Engelm........ .... IV HM

T. megacarpum Torr............................. V M

T. occidentale Gray............................. V M

Thermopsis montana Nutt................. IV, V, VI M

Thlaspi coloradense Rydb. (?)................... VII X

T. glaucum A. Nels. (?)........................ VII X

T. spp.—a new purple-flowered species............ VII X

Tithymalus luridus (Engelm.) Woot. & Small..... . III XM

Townsendia arizonica Gray.................. ..... I X

T. spp.. I X

Trifolium rydbergii Greene....................... VI M

T. repens Wheeler.............................. IV HM, R

Trisetum subspicatum (L.) Beauv................. IV M

Urtica breweri S. Wats.......................... IV HM

Valeriana ceratophylla (Hook.) Piper.............. IV HM

Veratrum speciosa Rydb...................... ..... V HM

Veronica alpina L............................ ... IV HM

V. americana Schwein............................ III HM
V. serpyllifolia L................................ IV HM
V. wormskjoldii Roem. & Schult................... V HM
Vicia americana Michx........................... IV M
Viola macloskeyi Lloyd or V. orbiculata Geyer....... V HM
V. nephrophylla Greene.......................... III HM
Woodsia oregana D. C. Eaton.................... III XM
Wyethia scabra Hook........................ .... I X
Zygadenus elegans Pursh..................... V, VI HM

Summary

1. An ecological survey was made of the vegetation of the region between the 111th and the 112th meridians west of Greenwich and the 38th and 39th parallels north of the equator. It lies northeast of Bryce Canyon and northwest of the Henry Mts.

2. The vegetation in this region has been little disturbed except to a small extent by lumbering and to a somewhat greater extent by grazing.

3. Studies were made from the rock desert regions in the eastern part of this quadrat up to the summits of the plateaus included within its borders, namely, Aquarius, Thousand Lake, Fish Lake, and Awapa of the High Plateaus and the monoclines known as the Water Pocket Flexure and Miner's Mountain.

4. The High Plateaus are glaciated, lava-capped uplifts due to faulting, whose summits are 11,000 feet and more in altitude. The Water Pocket Flexure and Miner's Mountain are monoclines of sedimentary strata which reach to 8250 feet in altitude and are dissected by numerous gorges.

5. The climate varies from desert in the lowlands at 5000 feet where the annual precipitation is 5 to 6 inches to alpine on the summits.

6. There is a wide variety of soils, both residual and transported.

7. The flora includes representative formations from southern desert to alpine scrub, the lowlands lying on the outskirts of Lower Sonoran territory and the summits on the tension line between the subalpine forest and the arctic-alpine desert.

8. The southern flora extends up the tributaries of the Colorado

system, but corresponding levels on the Great Basin slopes of the tables lack these species.

9. The timber line is determined by wind rather than by temperature, so the broader the summit the better the development of forest. Small tables and isolated peaks are occupied mostly by alpine scrub; larger tables, by parklike arrangements of scrub and forest.

10. As far as possible the various successions to the climax in each climatic zone were worked out.

Acknowledgment is made of the taxonomic aid furnished by A. S. HITCHCOCK in the determination of the grasses, by the late Dr. CHARLES C. PLITT in the identification of the lichens, by Dr. SEVILLE FLOWERS in that of the mosses and some of the vascular plants, and by Dr. AVEN NELSON and Mr. C. L. PORTER for determination of most of the vascular plants. Thanks are due to Dr. H. C. COWLES, who was my advisor throughout the progress of these studies, and to Dr. G. D. FULLER, who has offered valuable criticisms.

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LITERATURE CITED

1. ALTER, J. CECIL, Climatological data: Utah Section, U.S.D.A. Weather Bureau, 1930. 32: nos. 6, 7, 8. 1931. 33: nos. 6, 7, 8. 34: nos. 6, 7, 8.
2. BOWMAN, ISAIAH, Forest physiography. Wiley & Sons, New York. 1911.
3. DUTTON, C. E., The geology of the High Plateaus of Utah. Vols. I, II. Part of geological and geographical survey of the Rocky Mts. by POWELL and others. U.S. Geol. Survey. 1879.
4. RYDBERG, PER AXEL., Flora of the Rocky Mountains and adjacent plains. Published by author. 1922.
5. SAMPSON, ARTHUR W., Flora of Nevada and Utah. Pp. 24–31.
6. TIDESTROM, IVAR, Flora of Nevada and Utah. Contrib. U.S. National Herbarium. Vol. 25. 1925.

LIPASE PRODUCTION BY PENICILLIUM OXALICUM AND ASPERGILLUS FLAVUS[1]

DAVID KIRSH

Introduction

The fact is well established that the function of lipases, the group of enzymes hydrolyzing fats and oils, is as important in animal, plant, and microbial metabolism as are the other two large groups of enzymes, the proteases and carbohydrases. Among the different lipases, those produced by microorganisms, particularly the fungi, have been least studied and are therefore least understood, in spite of the fact that this subject had its beginning in 1897, when GERARD (5) reported the elaboration of this enzyme by a green species of *Penicillium*. Among the enzyme systems of fungi the carbohdrases and proteases have received most attention. Papers dealing with the enzymes acting on fats have been of a desultory nature, and, with few exceptions, concern themselves merely with the statement that certain fungi have been shown to possess the property of producing lipase (13).

Lipase production by *Aspergillus niger* has been found (4) to reach a maximum long after sporulation of the fungus, although TAKAMINE (12) and OSHIMA and CHURCH (8) report that enzyme production by *A. oryzae* on a solid medium is greatest just at the time of complete sporulation. The presence of coconut oil in the culture medium stimulates lipase production by various fungi (2), while the lipase content of koji (steamed rice infected with *A. oryzae*) was observed to be almost proportional to the fat content of the raw materials (6). *A. niger* (11) was also found to produce more lipase on a fat-containing medium than on a cane sugar or glycerol medium.

In the present investigation, solid media were employed in order to obtain a dry fungus lipase preparation similar to that of taka-

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diastase from *Aspergillus oryzae*, in quantities large enough to per-
mit a detailed study of the properties and mode of action of the
enzyme.

Experimentation

A series of fungi capable of growing upon fatty subtances were
isolated from various sources, namely, soil, sewage, forest organic
matter, decomposing Brazil nuts, etc., employing the customary en-
richment culture method (except in the case of the Brazil nuts, in
which the organisms were isolated directly) and plating out upon
Rahn's medium (3) containing olive oil. Water was added to the
source material if necessary to create optimum moisture conditions
and sufficient $(NH_4)_2HPO_4$ to give a final concentration of 0.25 per
cent. Rahn's medium was adjusted to neutrality and 0.1 per cent
brom thymol blue added. The enrichment culture material was
plated out and incubated at 28° C.; those colonies which grew rapid-
ly and produced sufficient acid from the olive oil to change the color
of the indicator from blue to yellow were selected for preliminary
study. Various species of *Aspergillus, Penicillium, Oidium, Mucor,
Trichoderma*, and *Monilia*, as well as several unidentified bacteria
and actinomyces, were thus isolated.

The lipolytic activity of the fungi was determined in the follow-
ing manner: 30 gm. of a substrate consisting of one part bran, one
part wood flour, three parts of a 1 per cent $(NH_4)_2HPO_4$ solution,
and sufficient olive oil to give a final concentration of 5 per cent,
were placed in pint Mason jars or some similar glass containers,
plugged with cotton, sterilized in intermittent flowing steam, and
inoculated with 3 cc. of a water suspension of spores. The spores
were obtained from a dried stock culture prepared in a similar
manner. The seed was used only after several generations of the
fungus originally grown on an agar medium had been cultured on
the solid medium, in order to allow the organism to adapt itself to
the substrate.

Since most workers agree that the quantity of extracellular en-
zyme is greatest just after complete fructification, the cultures were
analyzed after three to six days' incubation, during which time
sporulation was complete. The cultures were ground with sand and
80 cc. of distilled water, allowed to stand for one hour, and then

filtered into cheesecloth. Most of the water was removed in a squeez-ing device similar to a potato ricer, the rest of the water being ex-pressed in a small fruit press. The extract was centrifuged for 25–30 minutes, and an aliquot representing one-fourth (made up to 25 cc.) placed in a 250 cc. Erlenmeyer flask together with 1 cc. of 0.1 N H_2SO_4 (to adjust to approximately pH 5.0), 5 cc. of a pH 5.0 phos-phate buffer, and 5 cc. of a 50 per cent olive oil emulsion containing 2.5 per cent gum arabic (9). After the addition of 1 cc. of toluene, the flasks were stoppered, shaken, and incubated at 40° C. for 20 hours. The flasks, of which there were always two identical series: an un-boiled series designated in the tables under the heading Enzyme, and a boiled control series in which the extract was boiled for five minutes to destroy the enzyme, were titrated with 0.1 N NaOH against phenolphthalein after the addition of 75 cc. of 95 per cent alcohol and 25 cc. of ethyl ether. Lipolytic activity is arbitrarily expressed for comparative purposes as the number of cc. of 0.1 N acid split from the olive oil by the extract obtained as described. The details concerning the factors influencing the activity of the enzyme will be found elsewhere (7).

The two most active cultures of the many tested were found to be a form of *Penicillium oxalicum* (isolated from a deciduous forest soil using cod-liver oil in the enrichment culture), and a strain of *Aspergillus flavus*[2] (isolated from decomposing Brazil nuts); these organisms were employed in most of the subsequent studies.

LIPASE PRODUCTION ON A SOY BEAN MEDIUM.—In many of the early experiments employing the bran-wood flour-olive oil medium, no more than about 7 cc. of 0.1 N acid was split off from the olive oil by the enzyme, this figure representing about 9 per cent hydrolysis (5 cc. of the olive oil emulsion theoretically yields 78 cc. 0.1 N acid on complete hydrolysis when the saponification value of the oil is 190). In order to determine whether lipase production would be en-hanced if the organisms were grown on some natural oil-containing material, a medium was made up of 10 gm. of ground soy beans

[2] The writer is indebted to Dr. CHARLES THOM of the U.S. Department of Agriculture for the identification of these two species. Concerning the two organisms Dr. THOM writes, "The *Aspergillus flavus* is of the usual general type. The *Penicillium* has the spore-producing mechanism of *Penicillium oxalicum*, but a very woolly growing surface entirely different in general appearance from the form we commonly find in the soil."

(Harbinsoy variety containing 20.3 per cent oil), 10 gm. of bran, and
20 cc. of water. The fungi grew rapidly and luxuriantly, the extracts
hydrolyzing the olive oil to the extent of 35 per cent in one experi-
ment and 50 per cent in another. The results in table I, as in all sub-
sequent tables, are reported as cc. of 0.1 N acid, and indicate the
amount of cleavage brought about after 6 and 20 hours of incuba-
tion of the fungus extracts and olive oil.

TABLE I

LIPOLYTIC ACTIVITY OF EXTRACTS OF FUNGI GROWN ON
SOY BEAN MEDIUM (CC. OF 0.1 N ACID)

	HOURS					
	PENICILLIUM OXALICUM			ASPERGILLUS FLAVUS		
	0	6	20	0	6	20
Enzyme.................	....	57.3	74.8	....	66.0	77.8
Boiled control............	....	35.5	35.0	....	51 3	51.0
Activity.................	....	21.8	39.8	....	14.7	26.8
Unboiled control.........	35 3	38.8	40.0	50.0	54 5	56.5

The unboiled control differed from the boiled control in that the
enzyme was not destroyed by heat in the former and did not have
any oil emulsion added. The former control value is higher than the
latter since it allows for the action of the enzyme during the incuba-
tion period on oil extracted from the medium. For all practical pur-
poses, however, the boiled control value is used in calculating
enzymic activity, since this figure gives a measure of the total
enzyme present in the extract which can be obtained in a dry powder
form. For comparative purposes, the unboiled control values are
occasionally given.

By following· the changes in amino nitrogen content in the un-
boiled controls, it was definitely shown that the increase in titra-
table acidity in the enzyme flasks was not due to any protease action.
The slight increase in amino nitrogen over a period of 20 hours' in-
cubation corresponded to only about 2–3 cc. 0.1 N acid, a value
which is negligible when one considers the acidity resulting from the
action of the enzyme on the oil.

INFLUENCE OF OIL CONTENT OF MEDIUM.—To 30 gm. portions of the bran-wood flour medium was added sufficient olive oil to give concentrations ranging from 0 up to 30 per cent of the final weight. At a concentration of 20 per cent oil the physical condition of the medium was only fair, and at 30 per cent it was poor, being rather soggy owing to the large excess of oil. After four days' incubation the medium containing no oil had only a thin growth which failed to bind the substrate together. With increasing quantities of oil, the growth of the fungus was more abundant and the spores darker in

TABLE II

INFLUENCE OF CONCENTRATION OF OIL IN GROWTH SUBSTRATE
ON LIPASE PRODUCTION BY FUNGI (CC. OF 0.1 N ACID)

PERCENTAGE OIL ADDED TO MEDIUM	PENICILLIUM OXALICUM			ASPERGILLUS FLAVUS		
	ENZYME	CONTROL	ACTIVITY	ENZYME	CONTROL	ACTIVITY
0	35.8	24.0	11.8	30.4	22.8	7.6
2½	52.0	25.2	26.8	28.0	22.5	5.5
5	51.0	24.5	26.5	27.3	23.0	4.3
10	32.0	24.0	8.0	30.1	26.6	3.5
15	30.9	24.6	6.3	39.0	34.8	4.2
20	31.1	25.5	5.6	43.5	38.9	4.6
30	36.3	29.4	6.9	....	....	....

color, a compact mat being produced in concentrations of 5 per cent and upward. The extracts from the 20 per cent oil medium contained a slight amount of free oil after centrifuging, while the 30 per cent oil medium extract contained about 4 cc. of free oil. Aliquot portions of the oil were removed with the extracts in setting up the enzyme flasks.

The results in table II point to the fact that a medium containing from 2.5 to 5 per cent oil is most favorable; lipase formation by *Penicillium oxalicum* is greatest on this medium. The results are not so definite in the case of *Aspergillus flavus*, owing to its low activity; nevertheless they point to the same conclusion. It was first believed that the richer medium resulted in a greater production of heat by the organisms, this heat having a destructive effect upon the enzyme. When the experiment was repeated with the cultures incubated in a water bath at 28° C. (the advantage of this manner of

incubation is pointed out in a later experiment), the same general results were obtained.

The same type of experiment was employed with the soy bean medium using several varieties of soy beans having different oil contents, and ether-extracted beans to which definite quantities of soy bean oil were added. The results did not bring out any significant differences. It is possible that when different varieties of soy beans are employed the nature of the nutrients and the nitrogen content play important rôles in the metabolism of the organisms concerned. On the other hand, the actual differences in oil content were not

TABLE III

EFFECT OF PERIOD OF INCUBATION ON LIPASE PRODUCTION (CC. OF 0.1 N ACID)

INCUBATION PERIOD OF CULTURES IN DAYS	PENICILLIUM OXALICUM			ASPERGILLUS FLAVUS		
	ENZYME	CONTROL	ACTIVITY	ENZYME	CONTROL	ACTIVITY
2................	42.5	24.0	18.5	66 3	44.4	21.9
3................	55.6	26.3	29.3	73 0	51.1	21.9
4................	62.6	30.2	32 4	79.5	60.5	19.0
5................	53.2	29.4	28.8	78.3	59.9	18.4
6................	60.2	35 8	24.4	84.0	69 0	15.0
7................	58.0	34.1	23.9	86.2	67.5	18.7
10................	57.2	38.2	19.0	81.0	66.0	15.0
13................	61.5	45.4	16.1	89.0	76 6	12.4
17................	65.1	52.7	13.4	88.3	75.5	12.8
30................	60.5	53.2	7.3	85.0	73.3	11.7

great, since the extent was only from zero to 2 gm., whereas it went up to 12.9 gm. in the olive oil series.

EFFECT OF PERIOD OF INCUBATION.—As shown in table III, the lipase content reaches a maximum in both cultures after three to four days' incubation; after this it decreases slowly, so that after 30 days there is still an appreciable amount in the cultures. Fructification began after two days, and appeared to be complete after four days. The drying of the cultures on incubation may have had a preserving effect on the enzyme and thus account for its slow disappearance in the older cultures. In some large scale experiments, where greater quantities of medium were employed, fructification was considerably delayed.

EFFECT OF TEMPERATURE OF INCUBATION.—A series of jars was inoculated with *Penicillium oxalicum* and incubated in water baths at various temperatures. The water in the baths was kept constantly agitated by a small stream of air bubbles, thus causing a continuous circulation about the culture jars. A duplicate series was placed in a hot air incubator at 28° C., as well as in water at this temperature. The cultures were analyzed after four days' incubation while an extra series was kept at the lower temperatures for a longer period to permit fructification of the fungus. All the cultures were held at 28° C. for about 28 hours to permit good mycelial development,

TABLE IV

EFFECT OF TEMPERATURE OF INCUBATION OF PENICILLIUM
OXALICUM ON LIPASE PRODUCTION (CC. OF 0.1 N ACID)

INCUBATION TEMPERATURE (°C.)	INCUBATION 4 DAYS			INCUBATION 6 DAYS		
	ENZYME	CONTROL	ACTIVITY	ENZYME	CONTROL	ACTIVITY
13..................	39.2†	21.3	17.9	42.9§	23.4	19.5
18.5...............	55.5	29.3	26.2	50.8	31.5	19.3
24.................	69.5	35.0	34 5	71.6	33.4	38.2
28..	75.5	32.1	43.4	....	....	....
28 (air)*..........	59.5	27.7	31.8	....	....	....
35	62.5‡	29.5	33.0	....	....	....

* This set of cultures was kept in an electric hot air incubator; all the other sets were kept in water baths at the various temperatures.
† No sporulation in 4 days at this temperature.
‡ Poor sporulation.
§ This set of cultures was incubated for 8 days to allow sporulation.

after which time they were transferred to their respective water baths.

From table IV it appears that the greatest quantity of lipase is obtained when the fungus is incubated at 28° C. in a water bath. When kept at this temperature in air the yield is lower, owing to the heat generated by the growing organism. Air, a poor conducting medium, does not remove this heat as efficiently as does circulating water. In some cases the temperature of cultures placed in a 28° C. air incubator has been observed to go as high as 38° C., a temperature harmful to the enzyme. TAKAMINE (12) has reported temperatures as high as 42° C. in rooms where *Aspergillus oryzae* is originally

started at 30° C. for the manufacture of taka-diastase. In such cases the rooms are cooled to prevent destruction of the enzyme.

OPTIMUM CONCENTRATION OF SOY BEAN IN SUBSTRATE.—Jars of media were prepared in which the soy bean content was varied from 0 to 12.5 gm. with 2.5 gm. variation in each series. Correspondingly, the amounts of bran were varied so that the total quantity of solid material was 20 gm. When 12.5 gm. of soy bean was mixed with 7.5 gm. of bran, the resultant medium, after the addition of water, had poor physical condition, being soggy and pastelike. Two jars were also prepared containing 5 gm. of bran, 5 gm. of wood flour,

TABLE V

EFFECT ON LIPASE PRODUCTION OF VARYING RATIO OF SOY BEAN TO BRAN IN GROWTH MEDIUM OF PENICILLIUM OXALICUM (CC. OF 0.1 N ACID)

MEDIUM		ENZYME	CONTROL	ACTIVITY
BRAN (GM.)	SOY BEAN (GM.)			
20.0	0	49.8	30.7	19.1
17.5	2.5	64.3	32.6	31.7
15.0	5.0	66.9	31.7	35.7
12.5..	7.5	72.1	32.1	40.0
10.0	10.0	67.9	32.0	35.9
7.5......	12.5	69.9	35.7	34.2
5.0*	10.0	51.3	29.1	24.2

* Mixed with 5.0 gm. wood flour.

and 10 gm. of soy bean. The medium thus obtained was of a fine porous texture. The jars were inoculated with *Penicillium oxalicum.*

The results in table V indicate that a ratio of 7.5 gm. of soy bean to 12.5 gm. of bran yields the best medium for lipase production. When a still finer series was set up, employing quantities of soy beans ranging from 5 to 10 gm. at intervals of 1 gm., and correspondingly, quantities of bran ranging from 15 to 10 gm., no significant differences were obtained. In further experiments, therefore, the optimal ratio of ingredients was employed.

INFLUENCE OF VARIOUS PLANT MATERIALS IN SUBSTRATE ON LIPASE PRODUCTION BY PENICILLIUM OXALICUM.—A series of media containing ground cotton seeds, cotton seed meal, flax seeds, flax seed meal, soy beans, and soy bean meal, were inoculated with *P. oxali-*

cum. From the results in table VI, it appears that lipase production
is greatest on the soy bean medium. The seeds and the meals (seeds
from which most of the oil is removed by pressure) appear to serve
equally well as a substrate for enzyme production except in the case
of soy bean. Here the yield is twice as great on the bean as it is on
the meal. In a repetition of this experiment employing the soy bean
and the meal, the activity on the former was 26.3 cc. 0.1 N acid,
and on the latter 21.4 cc. 0.1 N acid, thus conforming more to the
results obtained with the other plant materials.

TABLE VI

INFLUENCE OF VARIOUS PLANT MATERIALS IN SUBSTRATE ON
LIPASE PRODUCTION BY PENICILLIUM OXALICUM
(CC. OF 0.1 N ACID)

PLANT MATERIAL IN SUBSTRATE	ENZYME	CONTROL	ACTIVITY
Cotton seeds...............	63.3	35.5	27.8
Cotton seed meal..........	69.3	41.4	27.9
Flax seed.................	61.5	37.0	24 5
Flax seed meal............	58.6	35.5	23.1
Soy beans.................	74.0	39.7	34.3
Soy bean meal.............	74.4	57.0	17.4
Soy beans*................	81.0	54.7	26.3
Soy bean meal*...........	81.0	59.6	21.4

* These results were obtained in a separate experiment.

PREPARATION OF A DRY FUNGUS LIPASE.—*Penicillium oxalicum*
was inoculated into 1600 gm. of moist soy bean medium. After five
days' incubation the cultures were ground and extracted twice with
water, the extracts being removed in an hydraulic press at 3000
pounds pressure per square inch. The combined extracts after cen-
trifuging were poured slowly into four volumes of 95 per cent alcohol
which was being agitated thoroughly by an electric stirrer. The pre-
cipitate was subjected to two more treatments with three volumes
of alcohol, and once to one volume of acetone. The enzyme was
filtered off and dried for several hours in an oven by means of a
current of air warmed to 40° C. From the activity of the extract
and the precipitated enzyme, it was calculated that slightly more
than 80 per cent of the lipase was recovered in the dry powder.

Table VIIA shows the activity of the dry enzyme together with that of a high lipase trypsin of pancreatic origin for comparative purposes. The activity of the preparation thus obtained was rather low, as was that of the extract of the culture. This may have been due to the fact that the culture was kept in an air incubator instead

TABLE VIIA

LIPOLYTIC ACTIVITY OF DRY ENZYME PREPARATIONS (CC. OF 0.1 N ACID)

Lipase employed	Quantity of lipase (mg)	pH	Enzyme	Control	Activity
Penicillium oxalicum	{ 125 250	5 0 5 0	18 4 22 8	13 0 14 0	5 4 8 8
High lipase trypsin	{ 125	5 0 8 0 8 9	26 6 27 0 42 6	13 0 2 7 35 2	13 6 24 3 7 4

TABLE VIIB

LIPOLYTIC ACTIVITY OF A DRY LIPASE OF PENICILLIUM OXALICUM (CC. OF 0 1 N ACID)

Quantity of lipase (mg)	Enzyme	Control	Activity	Hydrolysis of olive oil (%)*
125	28 3	13 1	15 2	19 49
250	37 4	15 0	22 4	28 72

* Not corrected for acidity resulting on incubating enzyme solution alone

of in a water bath, and also that it was removed before good sporulation had set in.

A similar preparation was later obtained from some cultures grown in 40 gm. lots of medium which gave five times as much enzyme on a yield times activity basis as the previous experiment. The degree of hydrolysis brought about by this preparation was three times as great as that obtained with the previous one (table VIIB).

A large scale experiment was recently attempted whereby 9.5 kg. of medium containing 51 per cent added moisture was inoculated

and kept in shallow layers in a water bath. The experiment was not conducted under pure culture conditions, it being assumed that a heavy inoculum would enable the fungus to grow quickly and crowd out any contaminants. The experiment proceeded successfully, and 380 gm. of dry enzyme was obtained, but unfortunately its lipolytic activity was very low.

It is of interest to note here that after four days' incubation there was a loss in dry weight of the culture of 31.5 per cent.

Discussion

It is apparent from an examination of the data that the activity of the extracts obtained in different experiments showed considerable variation. Thus the activity of *Penicillium oxalicum* extracts ranged from as high as 42.0 cc. 0.1 N acid down to 9 cc. These variations may be attributed to many things: the different lots of seed employed, variation in the amount of seed used for inoculation, etc. For this reason only results within the same experiment should be compared.

Many other results were obtained in this study which do not require presentation in tabular form. Thus hot water extraction of the wood flour or bran used in the olive oil medium did not effect lipase production, although washed bran yielded a lighter colored extract.

With the bran-wood flour-olive oil medium, asparagine, peptone, or KNO_3 offered no advantage over $(NH_4)_2HPO_4$ in the case of either organism. The addition of the mineral elements present in Rahn's nutrient solution for fat-splitting organisms exerted no beneficial effect. On the soy bean medium no additional minerals or nitrogenous materials were required. When $(NH_4)_2HPO_4$ was added to this medium in concentrations greater than 2 per cent of the dry medium, a diminution in lipase production was observed.

Where higher concentrations of olive oil in the bran-wood flour medium resulted in a lower yield of lipase, it is possible that the excess of metabolic by-products may have inactivated the enzyme or destroyed it as suggested earlier by DELEANO (1).

Although a very active dry lipase was not obtained during the course of the work, the fungus preparation did contain eight and

a half times more lipase per unit of protease than did the commercial pancreatic high lipase trypsin. The method therefore holds promise of yielding a preparation of comparatively high lipase and low protease activity. It remains to be seen why the organism fails to produce as active an enzyme when grown in larger quantities as it does in 40 gm. lots of medium.

Summary

1. *Penicillium oxalicum* and *Aspergillus flavus* produce a water soluble enzyme capable of hydrolyzing olive oil when grown on a bran-wood flour-olive oil medium or a bran-soy bean medium.

2. The most active extracts were obtained on the soy bean medium, which brought about as much as 50 per cent hydrolysis of the oil in 20 hours.

3. On the bran-wood flour-olive oil medium, the optimum concentration of the oil for lipase production lies between 2.5 and 5 per cent.

4. Lipase production is greatest after three to four days' incubation, or just at the time of complete sporulation.

5. Enzyme production is greatest when the cultures are incubated at 28° C. in a water bath. At this temperature in a hot air incubator, some of the lipase is apparently destroyed by the heat given off by the growing fungus.

6. A ratio of about 7.5 parts of soy bean to 12.5 parts of bran yields the best medium for lipase production.

7. Slightly more lipase is elaborated on a medium containing soy beans than on one containing soy bean meal. Flax seed and cotton seed do not result in as high a yield of lipase, although the meals produced from these seeds serve equally as well as the seeds themselves.

8. The enzyme precipitated from the *Penicillium oxalicum* extract by means of alcohol contained eight and a half times more lipase per unit of protease than did a commercial high lipase trypsin. The activity was low when the enzyme was obtained from large cultures; the dry enzyme prepared from 40 gm. lots of medium showed good activity.

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LITERATURE CITED

1. DELEANO, N. T., Zur Kenntnis der Desassimilation bei den Pflanzen. Biochem. Zeitschr. 17:225–230. 1909.

2. EYRE, J. C., Cultural studies on *Aspergilli*, with special reference to lipase production of strains isolated from stored copra and cacao. Ann. App. Biol. 19:351–369. 1932.

3. FRED, E. B., and WAKSMAN, S. A., Laboratory manual of general microbiology. McGraw-Hill, New York. 1928.

4. GARNIER, G., Lipase dans les cultures de quelques espèces d'*Aspergillus*. Compt. Rend. Soc. Biol. Paris 65:1490, 1583–1584. 1903.

5. GERARD, E., Sur une lipase végétale extraite du *Penicillium glaucum*. Compt. Rend. Acad. Sci. Paris 124:370–371. 1897.

6. ITO, M., Studies on the nature of koji-diastase. Jour. Faculty of Agr., Hokkaido Imperial Univ. 30:243–386. 1932.

7. KIRSH, D., Factors influencing the activity of fungus lipase. Jour. Biol. Chem. 108:421–430. 1935.

8. OSHIMA, K., and CHURCH, M. B., Industrial mold enzymes. Ind. and Eng. Chem. 15:67–70. 1923.

9. ROON, L., and OESPER, R. E., A contribution to the theory of emulsification based on pharmaceutical practice. Ind. and Eng. Chem. 9:156–161. 1917.

10. ROUGE, E., Le *Lactarius sanguifluus* Fr. et la lipase. Centralbl. Bakt. II. 18:403–417; 587–607. 1907.

11. SHENKER, R., Zur Kenntnis der Lipase von *Aspergillus niger* (van Tiegh.). Biochem. Zeitschr. 120:164–196. 1921.

12. TAKAMINE, J., Enzymes of *Aspergillus oryzae* and the application of its amyloclastic enzyme to the fermentation industry. Ind. and Eng. Chem. 6: 824–828. 1924.

13. WAKSMAN, S. A., Enzymes of microorganisms. Bact. Abst. 6:265–299; 331–360. 1922.

A NEW SPECIES OF CALAMOPITYS FROM
THE AMERICAN DEVONIAN[1]

DALE E. THOMAS

(WITH PLATES II, III)

Calamopitys eupunctata sp. nov.

Pith small, containing medullary tracheids and medullary xylem strands. Circum-medullary strands in a discontinuous ring. Leaf traces single at origin, becoming double farther out in the secondary xylem. All primary xylem strands centrally mesarch. Secondary xylem tracheids with multiseriate bordered pits on both radial and tangential walls. Pits evenly distributed, not grouped. Growth rings distinct; summer wood tracheids radially narrower and with thicker walls than those of the spring wood. Wood parenchyma abundant in the terminal position. Locally branched tracheids. Wood rays commonly uniseriate, frequently biseriate, broader rays occasional. Rays high.

Description of material

The plant material upon which this paper is based was collected by Professor L. C. PETRY, in October, 1924. The find was made in Taughannock Gorge, located on the west shore of Cayuga Lake, in central New York, about 10 miles from the upper or southern end of the lake. Stem fragments of this material were found near the foot of a rock fall on the north side of the gorge, about 100 yards below the main waterfalls. They were in one of the slabs of rock which held *Callixylon* remains described by ARNOLD (1). This slab had fallen from the Cashaqua Shale, exposed some 300 feet above the bed of the stream (18). The Cashaqua Shale belongs to basal Portage, a member of the Upper Devonian. It corresponds to the Sherburne flagstone of Ithaca as classified by WILLIAMS (22).

PETRY made a preliminary examination of sections which he cut from the specimen, and recognized that the material represented an

[1] This paper represents part of a thesis (18) presented to Cornell University, 1932.

undescribed form. Later he turned over to the writer all of this fossil material for preparation and description.

The material when found consisted of two pieces which matched along a plane oblique to the axis of the fossil. The specimen, as reconstructed, measured 75 mm. along its axis and had a maximum diameter of 30 mm. It tapered gradually to a point at one end and was broken obliquely at the other end.

The fossil was inclosed in a calcareous nodule, gray in color, with a slight reddish brown tinge. The fossil itself was black or nearly so, with marcasite filling the lumina of many of the cells. This iron sulphide was especially abundant in the wood rays of the fossil stem and has interfered to some extent with the preparation and interpretation of the material.

While the preservation is generally good for material of this type and age, nevertheless the specimen shows numerous local mineral replacements with a loss of cellular structure. Many of these regions probably represent the filling of cavities of local decomposition which antedated fossilization. These are most frequent in those parts of the stem originally occupied by softer parenchymatous tissues.

The description of this fossil material is based upon microscopic examination of the sections prepared by PETRY and also of a great number of sections prepared by the writer. Two types of slides were prepared,[2] cellulose peels and ground and polished sections. The collection of slides prepared from this stem includes 23 transverse sections, 12 tangential sections, and 14 radial sections. Many of these sections include only fragmentary material.

General anatomical structure

The outstanding features of a transverse section are: (1) the four parabola-like or fanlike groups of secondary xylem which include most of this tissue, (2) the prominent growth rings, and (3) the irregular shape of the pith (fig. 1).

PITH

The pith is rather indefinite as to its outer limits, extending between the wedges of the secondary xylem. The form is dependent

[2] For a description of the methods used in preparing the slides, see THOMAS (18), BARNES and DUERDEN (3), WALTON (20, 21), and KRÄUSEL (7).

upon the position of the leaf traces. In transverse section it is in general about 4 mm. long and 1.5 mm. broad, as measured to the circum-medullary primary xylem. It contains both primary xylem strands and a considerable number of more or less isolated tracheids. The parenchyma cells are not so well preserved; mineralization has been very pronounced wherever the cells formerly existed. These are typical parenchyma cells, short and with thin walls (fig. 2). The tracheids of the medullary strands show distinct sculpturing (fig. 3), as do the tracheids of the circum-medullary strands bordering the secondary wood.

The writer considers this stem protostelic, with much parenchyma accompanying the primary xylem. It may be considered as siphono-stelic since it has a definite pith with considerable parenchyma in which are located medullary strands and the more or less isolated tracheids. It is in reality a condition intermediate between a typical protostele and a typical ectophloic siphonostele.

PRIMARY XYLEM

The primary xylem is not a continuous mass of well organized tissue but consists of strands. Some of these strands, the medullary bundles, are located well within the pith; others, the circum-medullary strands, are in contact with the inner ends of the radial rows of secondary xylem elements. Radially broad strands lie between the medulla and each leaf trace. These radiating strands are sheetlike in form, some 10 to 12 tracheids wide in tangential direction, and with rows of secondary tracheids radiating out from each of them.

The circum-medullary primary xylem, in contact with the inner portion of the secondary wood, is 3 to 6 cells thick, an unusually thin layer. The medullary strands which are imbedded in the pith may be from 10 to 20 cells broad. Numerous medullary tracheids are found isolated in the pith; they also occur in small groups of from 3 to 6 elements. A longitudinal section shows that at least some of these medullary tracheids have connections with the medullary strands at a lower level. It is probable that these elements, which appear to be isolated as seen in cross sections, may be ramifications from the primary strands.

The centrally mesarch position of the protoxylem is illustrated in

figure 2, in a medullary bundle. The same condition may also be seen in the leaf trace bundles (figs. 4, 5). These illustrations also show that the centripetal and centrifugal tracheids are approximately the same size.

The protoxylem elements average 22 μ in diameter; metaxylem elements average 40 μ, with a maximum diameter of 55 μ observed. These are somewhat smaller than the tracheids of the secondary xylem, which average 50 μ and have an observed maximum diameter of 85 μ.

The markings on the walls of the protoxylem elements are in many places clearly visible. They are in all cases either annular or spiral (figs. 3, 6). The metaxylem includes only scalariform and pitted elements.

SECONDARY XYLEM

The secondary xylem constitutes the major part of the specimen (fig. 1). The arrangement of the secondary wood in four fan-shaped groups is characteristic throughout the specimen. This feature is associated with the outgoing leaf traces. The tracheids of this tissue range in diameter from 30 to 85 μ, with an average of 50 μ. The rows of tracheids are oriented in two ways: (1) in strictly radial rows, with the inner (proximal) portion of the rows well within the pith; (2) most of the rows are arranged in the four fan-shaped groups, the proximal portion of each row curving strongly toward the primary xylem in the radial axis of this group. The wood is traversed by numerous wood rays.

The walls separating the lumina of two adjacent tracheids of the secondary xylem average 9 μ in thickness. The thickness of the interluminal walls of the parenchymatous wood ray cells is less than 2 μ. The pits on the radial walls of the tracheids are evenly distributed. Alternate–multiseriate pitting predominates, with 3, 4, and 5 series the general rule. The pits are elongate in shape, measuring 9×3.5 μ, with the longer axis at right angles to that of the tracheid. The pits are full bordered (fig. 7).

One of the outstanding features of this stem is the tangential pitting, which is as clearly distinct as the pitting on the radial walls. Tangential pits are in shape, size, and arrangement like those of the

radial walls; frequently they are more scattered however (figs. 8, 9). In this stem, pits are present on all tangential walls and not solely on those of the terminal tracheids as in *Ginkgo* (5, p. 434) and some conifers.

Tangential pitting has been observed, in rare cases, in Paleozoic stems. It was first reported by SCOTT (9) for *Pitys antiqua*. Geologically this feature occurs as low as Middle Devonian, where it was reported by KIDSTON and LANG (6) in *Palaeopitys milleri*. Tangential pitting has also been reported in the following Paleozoic gymnosperms: *Callixylon trifilieve*, *C. newberryi* (2, 15), *Volkelia refracta* (17), *Mesoxylon multirame* (12), and *Bilignea resinosa* (13). These are all from the Carboniferous except *Palaeopitys milleri*, which is from the Middle Devonian and *Callixylon trifilieve* which is from the Upper Devonian.

SCOTT (9, p. 352) states that the pits do not cover the whole tangential surface of the tracheids in *Pitys antiqua*, nor are they as crowded as the pits on the radial walls. SCOTT (13) found the pitting on tangential walls of *Bilignea resinosa* differed from that on radial walls in being more widely scattered and in having much smaller borders. In *Mesoxylon multirame* tangential pitting is found only on a few tracheids. ARNOLD (2) reports that the pits on the tangential walls of the tracheids of *Callixylon newberryi* are smaller, more distantly spaced, and not grouped as are the radial pits.

The tangential pitting which resembles most that found in *Calamopitys eupunctata* has been described and photographed by KIDSTON and LANG (6) from *Palaeopitys milleri*. Their description applies to *C. eupunctata* except that they found the shape of the pits polygonal; those of *C. eupunctata* are oval. The polygonal shape is due to crowding, of course.

Another distinctive feature of *C. eupunctata* is the presence of branched tracheids. The branching occurs only in the tangential plane; it is frequent in regions where parenchymatous tissue is abundant and in connection with leaf traces (fig. 8). Evidence of tangential branching may be observed also in transverse sections (fig. 10). These show that branched tracheids occur not singly but in radial rows.

The writer has found no reference to branched tracheids in the

literature on fossil or on living gymnosperms. This irregularity in the form of some of the tracheids of the Taughannock specimen is a prominent characteristic in tangential sections.

The so-called "convoluted tracheids" of *Callixylon zalesskyi* described by ARNOLD (1) suggest a remote similarity to the branched tracheids. The convoluted appearance, however, seems not to include branching but only curvature and other distortions of the normal shape. There are a few places in the photomicrograph illustrating this feature where one might suspect branching.

SCOTT and JEFFREY (14, fig. 35) include a photomicrograph of a tangential section of *Archaeopitys eastmanii* which also shows a slight similarity to the branched tracheids. They use the term knots in referring to the features which they have illustrated. SCOTT (10), in describing similar "knots" observed in *Mesoxylon lomaxii*, states that in the outer secondary wood the position of a double leaf trace is marked, in tangential section, by the presence of two knots of irregularly interwoven tracheids, inclosing in their meshes the tracheids of the outgoing strand.

The "convoluted tracheids" and "knots" of other investigators may not be analogous to the "branched tracheids," but they are the only structures at all comparable with them.

Growth rings are pronounced in this specimen. The larger sections show four "annual rings." The difference in the tracheids in the spring and summer wood is twofold: a difference in the radial width of the elements and a difference in the thickness of their walls. The summer wood elements average, in radial breadth, 42 μ; those of the spring wood, 74 μ. The interluminal walls of summer wood elements average 10.5 μ; those of the spring wood elements, 8.3 μ. The most striking feature of the growth rings is the occurrence of parenchyma in the terminal position (figs. 11, 12, 13); this feature will be described later.

The presence of growth rings in Paleozoic fossil plants is only occasional although several cases have been reported. Among the species which show annual rings are *Callixylon erianum* (1), *Endoxylon zonatum* (13), *Bilignea resinosa* (13), and *Dadoxylon indicum* (4). The growth rings of the Taughannock specimen resemble most those of *B. resinosa*. This new species, however, differs in that it has

advanced to a higher state in the development of growth rings, with its terminal parenchyma.

The terminal parenchyma cells are radially much narrower, shorter, and with much thinner walls than the tracheids. Figure 13, a radial section, shows the short terminal parenchyma cells with the summer wood tracheids on the left and the spring wood tracheids on the right of the parenchyma cells. The terminal parenchyma constitutes a cylindrical sheet, with numerous patches of summer wood tracheids occupying from 15 to 20 per cent of the area. This cylinder is also interrupted by leaf trace windows. The terminal position of the parenchyma cells is established by the fact that these parenchyma elements are contiguous vertically with the narrow, thick walled, summer wood tracheids, and radially adjacent to the spring wood tracheids.

The presence of terminal parenchyma is the most advanced characteristic of this Upper Devonian stem. While terminal parenchyma is characteristic of some living genera of conifers, such as *Abies*, *Larix*, and *Tsuga*, it has not heretofore been reported in Paleozoic gymnosperms.

SCOTT (9) found isolated strands of xylem-parenchyma in *Pitys antiqua*, also in *Mesoxylon multirame* (12). JEFFREY (5) placed the origin of terminal parenchyma in the Jurassic. He states that parenchyma originated first in the terminal position in gymnospermous woods. Now, even though found much lower stratigraphically, it was found in the primitive position. It was also found in a woody stem with tangential pitting.

The tracheids are extremely variable in length, commonly 400–1000 μ long, the maximum measured being 1875 μ. Tangentially the ends of the tracheids are chisel-shaped. The end walls are perforated by typical bordered pits.

The wood rays are most frequently uniseriate; biseriate rays are common; broader rays are found. Uniseriate rays are commonly 5–9 cells high; broader rays vary from 10 to 25 cells high; one triseriate ray 30 cells high was observed (figs. 14, 15). The wood ray cells are all of one type, typical parenchyma cells. In some cases the wood ray lies between strictly parallel radial sheets of tracheids, a row of tracheids occupying the space between two vertically con-

secutive rays, the ends of the tracheids abutting on the marginal ray cells (fig. 15). Generally, however, the wood ray occupies a space between rows of tracheids which are contiguous above and below the rays. Tangential sections show both relationships.

LEAF TRACES

The arrangement of the secondary xylem in four fanlike sections is the most obvious feature in a transverse section of this stem. This characteristic is the result of the passing of leaf traces through the secondary xylem. Each of the transverse sections shows the four fans of secondary xylem. These fans include practically all of the secondary wood; only a few rows of tracheids lie in a strictly radial position, not inclined toward one of the circum-medullary protrusions in the axis of a leaf trace (fig. 1).

At no place in any of the sections is a leaf trace closer to the pith than 2 mm. The inner traces are always simple and centrally mesarch (fig. 4). Farther out the primary strand of the trace divides into two bundles (fig. 5). Still farther out and within the secondary xylem the trace assumes a direction perpendicular to the axis of the stem. Here a cross section of the stem shows a longitudinal section of the leaf trace with the two groups of protoxylem (fig. 6). The outermost traces are more complex.

The radial protrusion of circum-medullary primary xylem which extends distally to each leaf trace corresponds to the leaf gap of a siphonostelic stem. In the case of a siphonostele, parenchyma occupies the leaf gap in the axil of the trace; in this case primary xylem follows the outgoing trace. This radial protrusion is clearly shown in connection with the bottom leaf trace in figure 1. These narrow primary xylem wedges contain from 3 to 6 protoxylem points. This primary strand is in contact with an inner simple trace but is separated from the more distal traces by parenchyma tissue. There is no definite proof that a typical reparatory strand is formed but there is evidence favoring this interpretation. The double trace appears at times to have a reparatory strand just proximal to the trace and separated from both the trace and the radial protrusion by parenchyma.

Comparison of Calamopitys eupunctata with
other species of Calamopitys

The genus *Calamopitys* was described by UNGER (19) in 1856, founded upon *C. saturni* as the type species. Since then the following species have been added: *C. annularis* by SOLMS-LAUBACH (16), *C. beinertiana* and *C. fascicularis* by SCOTT (8, 9), *C. americana* by SCOTT and JEFFREY (14), and *C. radiata* by SCOTT (13).

C. americana and *C. eupunctata* have in common a mixed pith, mesarch xylem strands, high multiseriate rays, several rows of alternating pits on the radial walls of the tracheids of the secondary xylem, development of double leaf trace bundle from the single bundle (11, p. 209), arrangement of secondary xylem around the leaf trace, and form and character of the ray cells.

C. eupunctata does not check with the description of *C. americana* as reported by SCOTT and JEFFREY (14) on the following points:

1. The primary xylem ring of *C. eupunctata* does not show approximate continuity.

2. *C. eupunctata* has primary strands centrally mesarch.

3. Centripetal and centrifugal metaxylem elements of *C. eupunctata* are of the same size.

4. Tracheids of the secondary xylem are slightly larger than those of the metaxylem in *C. eupunctata;* in *C. americana* tracheids of the secondary xylem are smaller than those of the primary strands.

In addition to these exceptions, *C. eupunctata* shows the following characteristics not mentioned by SCOTT and JEFFREY (14) nor by SCOTT (11):

1. Arrangement of the bulk of the secondary xylem in four fan-shaped wedges.

2. Tangential pitting on the walls of all tracheids of the secondary xylem.

3. Distinct growth rings.

4. Abundant terminal parenchyma.

5. Presence of branched tracheids.

6. Wood rays commonly uniseriate.

Many of the items contrasted are quantitative and other characteristics observed in only one of these species may have been present in both species but observed in only one. The fact that most

of the points in which these two species are in disagreement have to do with the secondary xylem is significant.

C. eupunctata resembles *C. saturni* in having a discontinuous circum-medullary primary xylem and in having centrally mesarch primary strands. It differs from *C. saturni* in having medullary tracheids and medullary xylem strands, and in lacking the outward dilation of its wood rays. These two species differ also at all points in which the new species and *C. americana* differ, except in the form and arrangement of the circum-medullary bundles.

This new species has wood rays more like those of *C. annularis* than like those of *C. americana*, at least in so far as width is concerned. It is more closely allied with these three species than with *C. fascicularis* and *C. beinertiana*, the *Eristophyton* species of ZALESSKY (23). *C. eupunctata* shows no specific affinities with *C. radiata*, as described by SCOTT (13).

Summary

1. *C. eupunctata* shows all of the generic characteristics of *Calamopitys*, as summarized by SCOTT (11). It has a small pith surrounded by a ring of mesarch primary strands; it has a leaf trace which is single at its origin but divides farther out; it has considerable secondary xylem; the secondary and metaxylem tracheids have multiseriate bordered pits.

2. The characteristics which distinguish it from the other species of this genus have to do almost entirely with the secondary xylem. It seems expedient to classify this species under the genus *Calamopitys*. Even though the secondary xylem shows marked variations from that of other species of this genus, these are considered as characteristics of specific value rather than of generic value.

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LITERATURE CITED

1. ARNOLD, C. A., The genus *Callixylon* from the Upper Devonian of central and western New York. Papers Michigan Acad. Sci. Arts & Letters 9:1–50. 1930.

2. ———, On *Callixylon Newberryi* (Dawson) Elkins et Wieland. Contrib. Mus. Paleon. Univ. Michigan 3:207–232. 1931.

3. BARNES, B., and DUERDEN, H., On the preparation of celluloid transfers from rocks containing fossil plants. New Phytol. 29:74–76. 1930.

4. HOLDEN, RUTH, On the anatomy of two Palaeozoic stems from India. Ann. Bot. 31:315–326. 1917.

5. JEFFREY, E. C., The anatomy of woody plants. Chicago. 1917.

6. KIDSTON, R., and LANG, W. H., On *Palaeopitys Milleri*. Trans. Roy. Soc. Edinburgh 53:409–418. 1923.

7. KRÄUSEL, R., Die paläobotanischen Untersuchungsmethoden. Jena. 1929.

8. SCOTT, D. H., On the primary wood of certain Araucarioxylons. Ann. Bot. 13:615–619. 1899.

9. ———, On the primary structure of certain Palaeozoic stems with the *Dadoxylon* type of wood. Trans. Roy. Soc. Edinburgh 40:331–365. 1902.

10. ———, The structure of *Mesoxylon Lomaxii* and *M. Poroxyloides*. Ann. Bot. 26:1011–1030. 1912.

11. ———, Notes on *Calamopitys* Unger. Jour. Linn. Soc. London 44:205–232. 1918.

12. ———, The structure of *Mesoxylon multirame*. Ann. Bot. 32:437–457. 1918.

13. ———, Fossil plants of the *Calamopitys* type, from the Carboniferous rocks of Scotland. Trans. Roy. Soc. Edinburgh 53:569–596. 1924.

14. SCOTT, D. H., and JEFFREY, E. C., On fossil plants showing structure, from the base of the Waverly Shale of Kentucky. Phil. Trans. Roy. Soc. London 205:315–373. 1914.

15. SEWARD, A. C., Plant life through the ages. Cambridge. 1931.

16. SOLMS-LAUBACH, H., Graf zu, Über die Seinerzeit von Unger beschriebenen strukturbietenden Pflanzenreste des Unterculm von Saalfeld in Thüringen. Abhandl. könig. Preuss. Geol. Landsanst. 23:63–77. 1896.

17. ———, Ueber die in den Kalksteinen des Kulm von Glätzisch-Falkenberg in Schlesien enthaltenen structurbietenden Pflanzenreste. IV. Zeitschr. Bot. 2:1910.

18. THOMAS, D. E., A new American species of *Calamopitys* from the Devonian. Thesis, Cornell University. 1932.

19. UNGER, F., Schiefer- und Sandsteinflora. Denkschriften der Kaiserl. Akad. Wiss. Wien 11:139–186. 1856.

20. WALTON, J., A method of preparing sections of fossil plants contained in coal balls, or in other types of petrifaction. Nature 122:571. 1928.
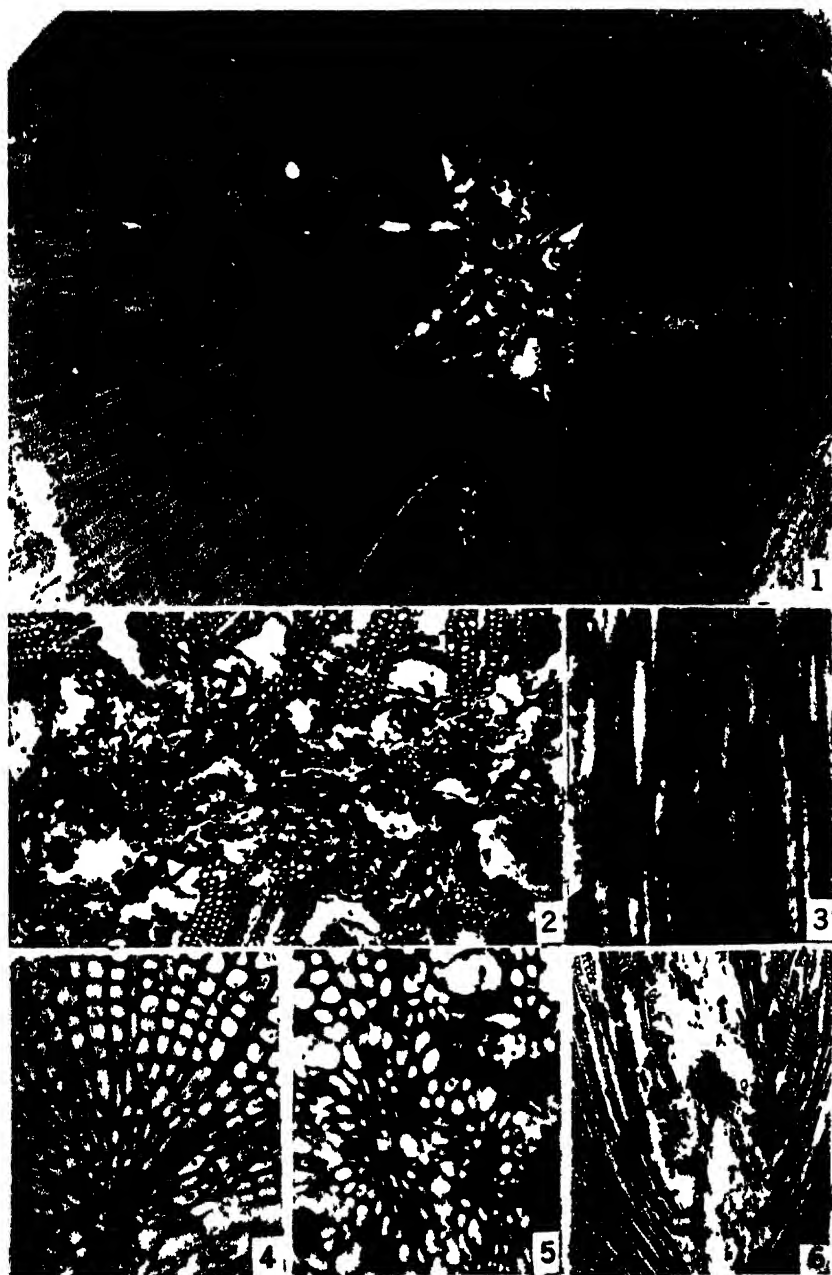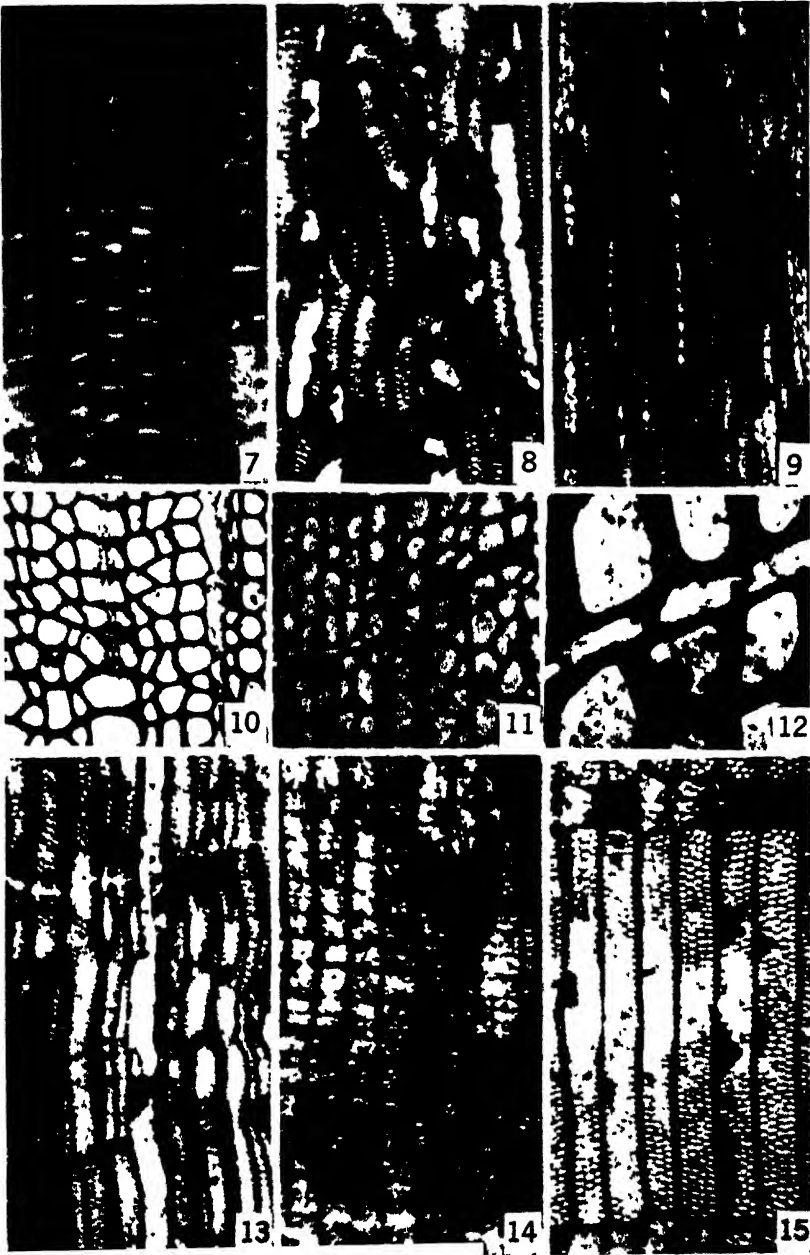
21. ———, Improvements in the peel-method of preparing sections of fossil plants. Nature 125:413. 1930.

22. WILLIAMS, H. S., Watkins Glen-Catatonk. Folio 169. Atlas of the United States. U.S. Geol. Survey. 1909.

23. ZALESSKY, M. D., Étude sur l'anatomie du *Dadoxylon Tchihatcheffi*. Goeppert sp. Mem. Comité Geol. Russe. N.S. 68:29. 1911.

EXPLANATION OF PLATES II, III

All of the figures are photomicrographs from untouched negatives.

FIG. 1.—General transverse section showing pith, arrangement of secondary xylem, position of leaf traces, and growth rings. ✕7.

FIG. 2.—Central part of fig. 1 enlarged to show pith, medullary and circum-medullary strands, and arrangement of proximal portion of secondary xylem. ✕25.

FIG. 3.—Radial section through medullary bundle showing sculpturing of elements. ✕100.

FIG. 4.—Transverse section showing simple leaf trace. ✕100.

FIG. 5.—Transverse section showing double leaf trace with two protoxylem groups. ✕100.

FIG. 6.—Transverse section (cellulose-peel) showing more advanced stage of double trace; section through primary bundle of trace nearly longitudinal. ✕15.

FIG. 7.—Radial section showing pitting of tracheids of secondary xylem. ✕450.

FIG. 8.—Tangential section showing branched tracheids accompanied by numerous wood rays. ✕80.

FIG. 9.—Tangential section showing wood rays of various widths and heights; pitting on tangential walls of tracheids. ✕100.

FIG. 10.—Transverse section showing pitted radial bands crossing some tracheids which are tangentially broad; each band represents the crotch of a branched tracheid. ✕100.

FIG. 11.—Transverse section through growth ring; terminal parenchyma running diagonally from left to right; summer wood elements above parenchyma. ✕100.

FIG. 12.—Transverse section showing portion of fig. 11 enlarged; summer wood elements above parenchyma. ✕450.

FIG. 13.—Radial section showing vertical row of short, thin walled parenchyma cells in terminal position, with summer wood elements on left, spring wood elements on right. ✕100.

FIG. 14.—Radial section showing exceptionally high wood ray; shape of ray cells and pits visible. ✕100.

FIG. 15.—Radial section showing row of tracheids abutting on wood ray above and below; both rays are low. ✕100.

MACROSPOROGENESIS AND DEVELOPMENT OF THE EMBRYO SAC OF LILIUM HENRYI[1]

D. C. COOPER[2]

(WITH PLATES IV, V AND NINE FIGURES)

Until recently, the type of development of the embryo sac in the Liliaceae has been considered one of the simplest to be found among the angiosperms. According to the common conception of the so-called "lily" type, the archesporial cell without division becomes the macrospore mother cell. As a result of three nuclear divisions and later cell divisions, a typical 8-nucleate, 7-celled embryo sac is formed. BAMBACIONI (1, 2), however, in his study of the development of the embryo sac of *Fritillaria persica*, found that four nuclear divisions actually intervene between the archesporial cell and the egg. In the course of the third division, three of the four nuclei formed as a result of the second division migrate to the chalazal end of the embryo sac, leaving one nucleus in its micropylar region. All the nuclei then undergo mitosis. The three chalazal spindles unite to form a common spindle which bears 3n chromosomes, whereas the micropylar spindle bears n chromosomes. As a result of this division the embryo sac is again 4-nucleate, two nuclei being haploid and two triploid. One further nuclear division occurs. BAMBACIONI and GIOMBINI (3) describe a similar process in the formation of the female gametophyte of *Tulipa gesneriana*, and later BAMBACIONI-MEZZETTI (4) reports the same type of embryo-sac development for *Lilium bulbiferum*, *L. candidum*, and *Tulipa praecox*. The writer (6), in a preliminary paper, briefly described a

similar method of embryo-sac formation in *Lilium henryi* and noted that this method is also found in *L. speciosum*, *L. philadelphicum*, and *L. longiflorum* var. *eximium* (*L. harrisii*).

Material and methods

Buds of various ages, open flowers, and young fruits of *Lilium henryi* Baker were collected from plants growing in the gardens of the Department of Horticulture at the University of Wisconsin. The ovaries were dissected out, cut transversely in small pieces, and either fixed in Carnoy's solution or dipped in that solution for a few seconds and then placed in Karpechenko's modification of Navashin's fluid. Good preparations showing stages in the development of the embryo sac were obtained from material in both fixatives. Material of *L. regale* Wilson was similarly fixed for a comparative study. The material was sectioned at 12–18 μ and stained in dilute Delafield's haematoxylin.

Preparations of ovaries of *Lilium* used for class work in the Department of Botany were also examined. These include material of *L. speciosum* Thunb., *L. philadelphicum* Linn., and *L. longiflorum* Thunb. var. *eximium* Nichols (*L. harrisii* Carr.). The history of embryo-sac development was consistent in all the material examined. The figures and description contained herein refer to *L. henryi* unless otherwise stated.

Investigation

DEVELOPMENT OF EMBRYO SAC

In *L. henryi* the primary archesporial cell functions as the embryo sac mother cell. During the heterotypic prophases this cell is about twice as long as broad and is somewhat flattened longitudinally, the nucleus being in the mid-region of the cell. The axis of the spindle of the first meiotic division is approximately longitudinal (fig. 10).[3] Twelve pairs of chromosomes can be counted on the equatorial plate. No cell plate is formed after this division. The second division takes place almost immediately, binucleate stages being relatively scarce in the preparations. Twelve chromosomes can be seen on each of the two equatorial plates in figure 11. The axes of the homoeotypic spindles may be parallel or at an oblique angle to each other. COULTER (7) reported that the micropylar spindle of *L. philadelph-*

[3] See plate IV, facing page 354.

icum in this division is transverse, the antipodal one longitudinal. His figures, however, indicate that he was describing the third rather than the second division, since a greater number of chromosomes appear on the chalazal spindle.

Four nuclei are formed as a result of the homoeotypic division (fig. 12). These nuclei usually lie approximately in a row, evenly distributed along the length of the sac. The micropylar nucleus remains at that end of the sac and the other three nuclei migrate to positions near the opposite end (fig. 13). All four now prepare to divide (fig. 14). SARGANT (11, 12) noted indications of spindle fibers surrounding the well differentiated naked spiremes in the 4-nucleate embryo sac of *L. martagon*. These spiremes were always arranged in the same way: a single nucleus near the micropyle and a group of three at the chalazal end. Since she was unable to find 8-nucleate embryo sacs in the neighboring ovules, she did not consider that the division thus indicated had any special significance. Her figure 36 shows the four nuclei in a late spireme with a bipolar spindle at the micropylar end of the embryo sac and a tripolar one at the opposite end. This is probably a prophase stage of the third division.

The nucleus at the micropylar end of the embryo sac of *L. henryi* divides in the usual manner. The division spindle is often in a transverse position (figs. 16, 18, 20, 21, 23, 24, 26), but it may be longitudinal (figs. 19, 27) or at an oblique angle (figs. 17, 25) to the long axis of the sac.

The three chalazal nuclei form spiremes (fig. 13), their membranes disappear (fig. 14), and soon a spindle is formed for each nucleus (text fig. 1; figs. 15, 16). In the chalazal portion of the embryo sac shown in figure 1 and figure 15, the chromosomes of each nucleus are arranged in a discontinuous spireme and the nucleolus is still present in the central region. The spindles formed in connection with these three nuclei now unite in such a manner that a single multipolar spindle is formed (fig. 4) which ultimately becomes bipolar (fig. 5). During this rearrangement the chromosomes become so shifted and rearranged (text figs. 2, 3; figs. 17–22) as to form a common equatorial plate. COULTER (7) noted multipolar spindles in *L. philadelphicum* (his figs. 9 and 11), but thought this to be an unusual, possibly a pathological condition.

The single equatorial plate now contains 36 chromosomes (fig.

6 *b*, fig. 24). The spindle is usually arranged longitudinally in the embryo sac (figs. 23, 25, 26), but may be transversely oriented (fig. 24). The embryo sac now again contains two spindles (figs. 23–26), but at this stage there are 12 chromosomes on the micropylar and 36 on the chalazal spindle (fig. 24). COULTER'S figures 3, 4, 5, 9, 10, 11, and 12 show more chromosomes on the chalazal spindle than are
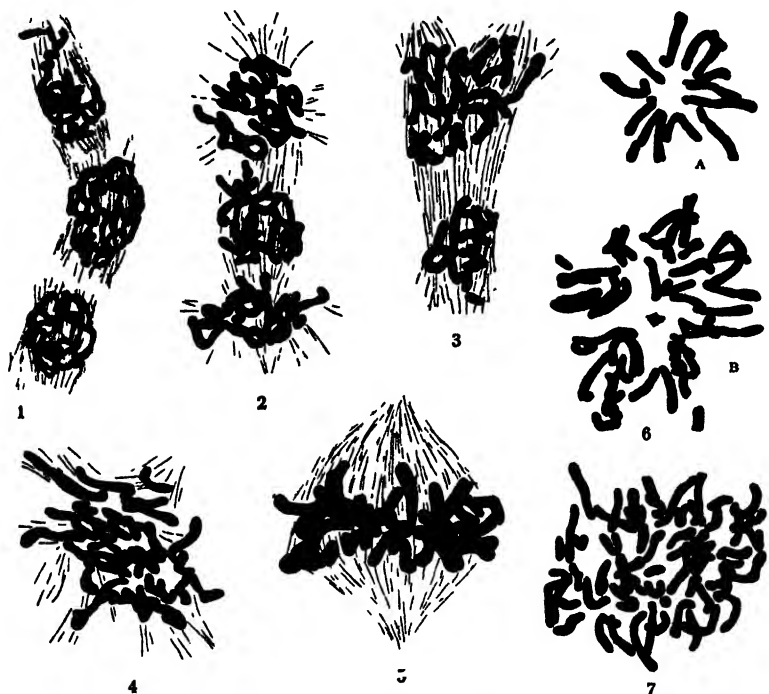
FIGS. 1–7.—Figs. 1–3, stages in fusion of three chalazal spindles in embryo sacs whose nuclei are undergoing third division. Fig. 4, polar view of multipolar spindle in chalazal end. Fig. 5, lateral view of bipolar spindle formed as result of fusion of spindles. Fig. 6: *A*, polar view of micropylar equatorial plate showing 12 chromosomes; *B*, polar view of chalazal equatorial plate with 36 chromosomes. Fig. 7, polar view of equatorial plate in endosperm cell; 60 chromosomes (5n) present. All ×750.

to be found on the micropylar spindle of what he considered the homoeotypic division. Four nuclei are again present at the end of this third division; two large nuclei lie in the chalazal region, and two smaller ones in the micropylar region (figs. 27, 28). The spindles of the third division are more or less persistent. GUIGNARD (8) noted a curious difference between the numbers of chromosomes present

respectively in the micropylar and chalazal nuclei in the embryo sac of *L. candidum*. MOTTIER (9) likewise observed a greater number of chromosomes on the chalazal spindle, but considered this condition to be due to an increased amount of chromatic material formed as a result of growth.

Up to this time the cytoplasm has been finely vacuolate, and no large central vacuole is present in the mid-region of the sac. The sac now grows and elongates, coming to be about four times as long as broad. Vacuoles (fig. 28) appear in the cytoplasm in the mid-region; these may unite to form a large central vacuole. In some instances, however, the central vacuole is not conspicuous until after the last nuclear division (fig. 38).

A densely staining mass of cytoplasm appears at each end of the spindles of the third division, in close contact with the nuclei (fig. 27). These masses are more prominent and have the appearance of a felted pad applied to one side of the nucleus in the case of *L. speciosum* (fig. 28). Remnants of the spindles remain in the cytoplasm between these pads. COULTER showed the remnants of such spindles in his figure 6. He considered this the 4-nucleate stage resulting from the homoeotypic division, but because of the difference in the size of the nuclei it appears probable that he had a 4-nucleate stage following the third division.

Three of the four nuclei now present, the two smaller (n) ones at the micropylar end of the embryo sac and one of the 3n nuclei in the chalazal region, undergo typical mitoses (fig. 29). Many more chromosomes are present on the chalazal than on either micropylar spindle. COULTER in his figures 13, 14, and 15 showed more chromosomes on the chalazal spindles than on the micropylar spindles of the last division.

The other 3n nucleus, near the chalazal end of the embryo sac, undergoes a more or less abortive division. Although there seems to be no regular formation of chromosomes, a spindle is formed and granules or masses of chromatic material pass to each pole. This nucleus is in a reticulate condition at the time its sister nucleus contains a spireme (fig. 30). Later a spindle is formed, and chromatic material or aggregations of this material are to be seen on the spindle (figs. 31–33). In some instances bodies resembling chromosomes are present (fig. 33). These pass irregularly to the poles and two small,

somewhat irregularly shaped nuclei are formed simultaneously with the development of normal nuclei in other portions of the embryo sac (figs. 34, 35). There is no evidence that direct division of this second chalazal nucleus ever occurs. An examination of SARGANT's (11) figures of amitosis in *L. martagon* shows stages similar to those herein described. MOTTIER likewise figured a spindle in connection with the abortive division of the chalazal nucleus of the same species.

In one case observed, only three spindles were to be seen at the time of this last division (fig. 36). In this instance, however, about 70 chromosomes could be counted on the chalazal spindle (fig. 37). This would seem to indicate that in this instance the two chalazal spindles of the last division, like those of the third division, had united into a common spindle.

During the prophases of the fourth division, the felted pads found at the sides of the nuclei spread out into perinuclear zones, so that, at the equatorial plate stage, each spindle is practically surrounded by a zone of dense cytoplasm (fig. 29). The remnants of the spindles of the preceding division persist between the respective perinuclear zones. At the conclusion of the fourth division the four nuclei at each end of the embryo sac are held together by two prominent spindles and a less prominent one (figs. 34, 35). Cell plates are formed across these three spindles in such a manner that three cells are formed at each end of the sac, leaving two nuclei, one small (n) and one large (3n), in the large central cell (figs. 35, 38). The formation of a cell plate across the spindle nearest the chalazal end of the sac is further evidence that the abortive division at this end is essentially mitotic rather than amitotic (fig. 34). COULTER in his figures 14 and 15 showed remnants of the spindles of the preceding division in *L. philadelphicum* still extant at the anaphase and telophase stages of the final division.

Stages in fertilization (fig. 39) show the union of one male gamete nucleus with the egg nucleus to form the zygote (figs. 39, 40). The other male gamete nucleus unites with the two polar nuclei, one large (3n) and one smaller (n), to form the primary endosperm nucleus (figs. 39, 41). The male gamete nucleus may be easily identified by its shape. SAX (13) figured the fusion of one large and two smaller nuclei in the formation of the endosperm mother cell of *Fritillaria pudica*, as does also NOTHNAGEL (10) in the case of

Lilium martagon. BLACKMAN and WELSFORD (5) figure a similar
fusion of nuclei in both *L. martagon* and *L. auratum.* Sixty chromo-
somes (5n) are present on the equatorial plates of dividing endo-
sperm nuclei (fig. 7). Such a number is to be expected as a result of
the union of nuclei herein described. The relationship between em-
bryo (2n), endosperm (5n), and nucellus (2n) differs markedly from
the 2-3-2 relationship usually found in angiosperms.
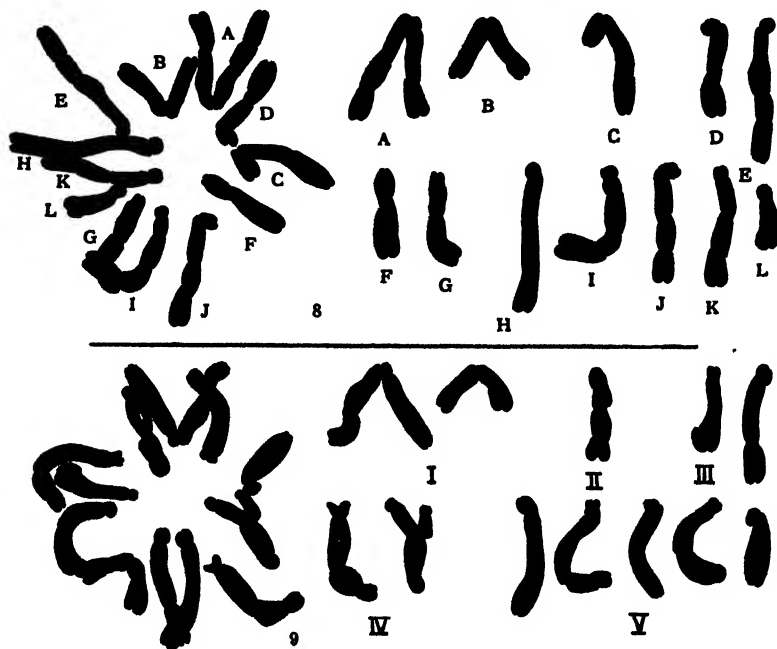
FIGS. 8, 9.—Fig. 8, polar view of haploid equatorial plate in third nuclear division in
embryo sac. At right, chromosomes *A–L* assorted into 5 groups according to size and
shape. Fig. 9, polar view of haploid equatorial plate in last nuclear division in embryo
sac. At right chromosomes arranged in 5 groups according to size and shape. All
×1450.

CHROMOSOME MORPHOLOGY

Polar views of the haploid equatorial plates of the third and fourth
nuclear divisions in the formation of the embryo sac are ideal for a
study of the shape and individuality of the chromosomes. Five types
of chromosomes are distinguishable at this stage. The first is a
V-shaped element with the spindle-fiber attachment zone in its mid-
portion (fig. 9 I). The larger of the two chromosomes of this type

shows approximately a 20 per cent difference in length between its
arms (fig. 8 A). The longer arm has a secondary constriction about
one-third its length from the distal end. The shorter arm has a simi-
lar constriction closely adjacent to the attachment zone. The smaller
V-shaped chromosome has a median attachment region (fig. 8 B).
The second type of chromosome, of which only one occurs, is J-
shaped (fig. 9 II). The attachment region is so located that one
arm is approximately three times the length of the other (fig. 8 C).

A third type of chromosome has a rather large lobe beyond the
attachment region and a secondary constriction about one-third of
its length from the opposite end (fig. 9 III). The two chromosomes
of this type differ markedly in length (fig. 8 D, E). A fourth type
(fig. 9 IV), represented by two chromosomes, is rod-shaped with a
small subspherical head end (fig. 8 F, G). A fifth type has a long
shaft with a rounded lobe beyond the attachment region (fig. 9 V).
The five chromosomes of this pattern range in length so that the
longest is twice the length of the shortest (fig. 8 H–L).

Summary

1. The egg is removed from the macrospore mother cell of *Lilium
henryi* by four divisions instead of three as has usually been de-
scribed for members of this genus.

2. The nucleus of the macrospore mother cell and its daughter
nuclei pass through the heterotypic and homoeotypic divisions re-
spectively without cell division, so that the embryo sac is 4-nucleate.

3. Three of these nuclei pass to the chalazal end of the embryo
sac; all four nuclei divide simultaneously.

4. The spindles of the three chalazal nuclei unite and a single bi-
polar spindle bearing 36 chromosomes is formed.

5. As a result of the third division the embryo sac is again 4-
nucleate, two haploid nuclei being at the micropylar end and two
triploid nuclei at the chalazal end.

6. Three of these four nuclei, the two with n chromosomes and one
with 3n, pass through typical mitoses; the other chalazal nucleus
divides in an abortive manner.

7. The cells of the embryo sac are formed as a result of cell-plate
formation across the spindle of this last (fourth) division as well as
across the persistent spindles of the preceding division.

8. In the process of fertilization one male gamete nucleus unites with the egg nucleus to form the zygote nucleus (2n) and the other male gamete nucleus unites with the two polar nuclei, one small (n) and one large (3n), to form a 5n primary endosperm nucleus.

9. Sixty chromosomes are present on equatorial plates of dividing endosperm nuclei.

10. An examination of preparations showing embryo-sac development in *L. speciosum*, *L. philadelphicum*, *L. longiflorum* var. *eximium* (*L. harrisii*), and *L. regale* reveals a history similar to that here described for *L. henryi*.

11. The history of embryo-sac development reported here for five species of *Lilium* is essentially the same as that described by BAMBACIONI for other members of the Liliaceae. It is very probably the characteristic lily type.

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LITERATURE CITED

1. BAMBACIONI, V., Come avviene in *Fritillaria persica* L. lo sviluppo del gametofito femminile e l'aumento dei cromosomi nella regione chalazale. Rend. acc. Lincei Roma, cl. fis. Mat. Nat. ser. 6. 6:544–546. 1928.

2. ———, Ricerche sulla ecologia e sulla embriologia di *Fritillaria persica* L. Annali di Bot. 18:7–37. 1928.

3. BAMBACIONI, V., and GIOMBINI, A., Sullo sviluppo del gametofito femminile in *Tulipa Gesneriana* L. Annali di Bot. 18:373–386. 1930.

4. BAMBACIONI-MEZZETTI, V., Nuove ricerche sull'embriologia delle Gigliacee. Annali di Bot. 19:365–368. 1932.

5. BLACKMAN, V. H., and WELSFORD, E. J., Fertilization in *Lilium*. Ann. Bot. 27:111–114. 1913.

6. COOPER, D. C., Development of the embryo sac of *Lilium Henryi*. Proc. Nat. Acad. Sci. 20:163–166. 1934.

7. COULTER, J. M., Contribution to the life history of *Lilium philadelphicum*. I. The embryo sac and associated structures. BOT. GAZ. 23:413–422. 1897.

8. GUIGNARD, LÉON, Nouvelles études sur la fécondation. Ann. Sci Nat. Bot. ser. VII. 14:163–296. 1891.

9. MOTTIER, D. M., Ueber das Verhalten der Kerne bei der Entwicklung des Embryosackes und die Vorgänge bei der Befruchtung. Jahrb. Wiss. Bot. 31:125–158. 1898.

10. NOTHNAGEL, MILDRED, Fecundation and formation of the primary endosperm nucleus in certain Liliaceae. BOT. GAZ. 66:143–161. 1918.

PLATE V
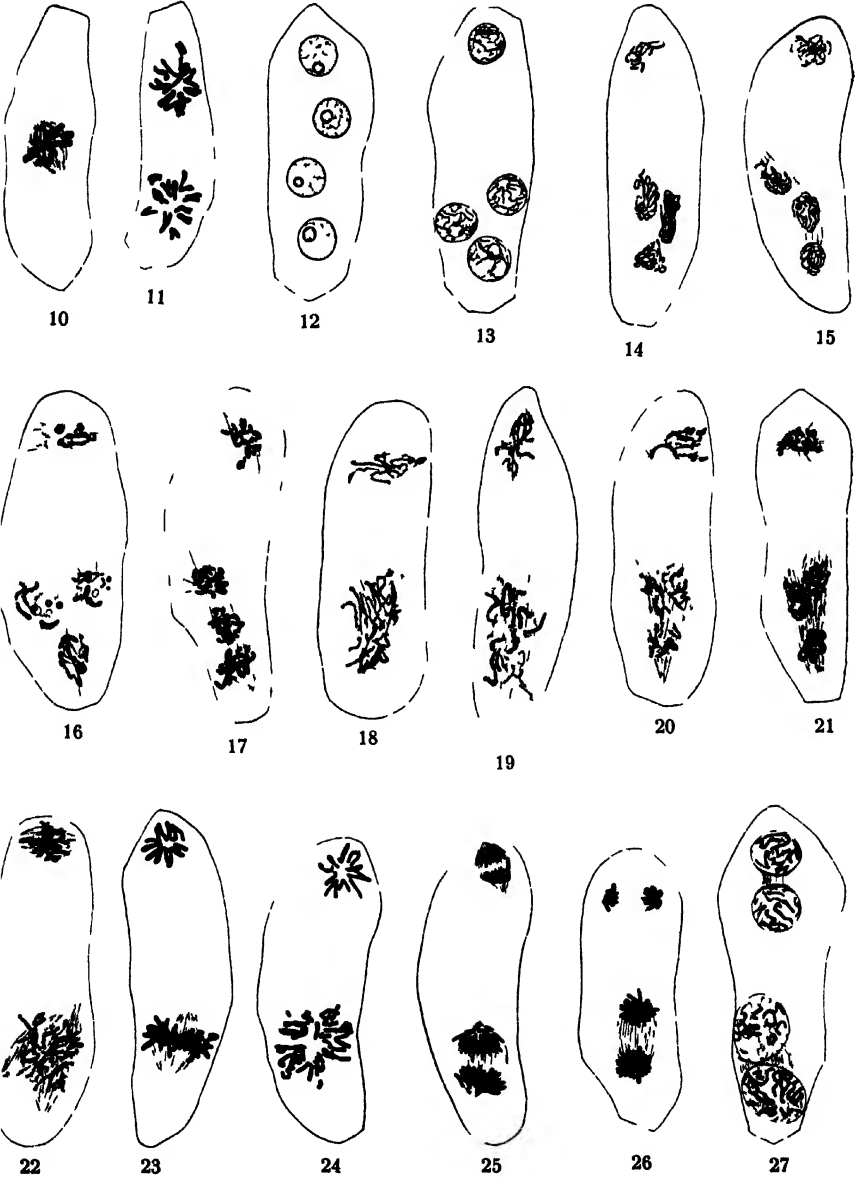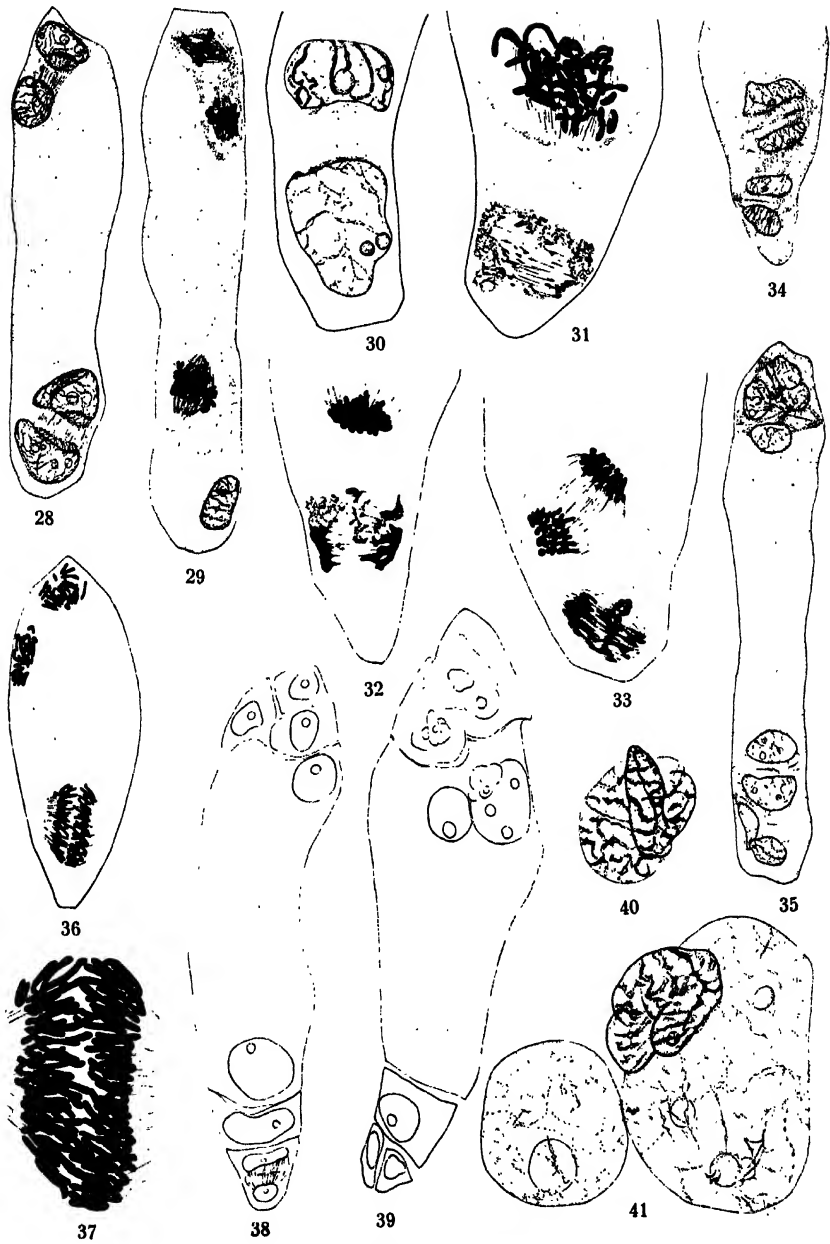
11. SARGANT, ETHEL, Direct nuclear division in the embryo sac of *Lilium Martagon*. Ann. Bot. 10:107–108. 1896.

12. ———, The formation of the sexual nuclei in *Lilium Martagon*. I. Oogenesis. Ann. Bot. 10:445–477. 1896.

13. SAX, KARL, Fertilization in *Fritillaria pudica*. Bull. Torr. Bot. Club 43: 505–522. 1916.

EXPLANATION OF PLATES IV, V

All figures unless otherwise stated were drawn at a magnification of 480× and are reduced approximately one-half in reproduction. All figures are so arranged that the micropylar end of the embryo sac is toward the top of the page.

PLATE IV

FIG. 10. Macrospore mother cell of *L. henryi*. Lateral view of heterotypic equatorial plate.

FIG. 11. Polar view of homoeotypic equatorial plates.

FIG. 12. Four-nucleate embryo sac after homoeotypic division.

FIG. 13. Same, later stage. Three nuclei migrated to chalazal region of sac.

FIG. 14. Same. Nuclear membranes disappeared.

FIG. 15. Early spindle development, third nuclear division.

FIGS. 16–22. Stages in union of the three chalazal spindles. Orientation of micropylar spindle also shown.

FIG. 23. Bipolar chalazal spindle.

FIG. 24. Polar view of two spindles of third division, with 12 chromosomes present on micropylar and 36 on chalazal spindle.

FIGS. 25, 26. Anaphase and telophase stages of third division.

FIG. 27. Four-nucleate embryo sac after third division, showing two small and two large nuclei and persistent spindles.

PLATE V

FIG. 28. *L. speciosum*, somewhat later stage than figure 27, showing cytoplasmic pads on adjacent surfaces of nuclei as well as persistent spindles.

FIG. 29. *L. henryi*, equatorial plates, last (fourth) nuclear division in embryo sac.

FIGS. 30–33. Stages in abortive division of chalazal nucleus. ×850.

FIG. 34. Cell-plate formation at chalazal end of embryo sac.

FIG. 35. Cell-plate formation at both ends of embryo sac.

FIG. 36. Fourth nuclear division, showing large spindle in chalazal region.

FIG. 37. Chalazal spindle from figure 36. About 70 chromosomes passing to each pole. ×1450.

FIG. 38. Eight-nucleate, 7-celled embryo sac.

FIG. 39. Embryo sac showing nuclear unions.

FIG. 40. Male gamete nucleus closely appressed to egg nucleus (from fig. 39). ×2900.

FIG. 41. Male gamete nucleus in close association with two polar nuclei (from fig. 39). ×2900.

ANATOMY OF THE SEEDLING OF ASPARAGUS OFFICINALIS

NAOMI MULLENDORE

(WITH FORTY-FOUR FIGURES)

Introduction

Of the works published concerning *Asparagus officinalis* L., some, such as those of ROBBINS (11) and JONES and ROSA (9), have dealt chiefly with the cultural and commercial aspects rather than with the morphological ones. BORTHWICK (4), ROBBINS and BORTHWICK (12), COOLEY (5), and BLASBERG (3) have used morphology largely as a basis for physiological study. Some of these have dealt with limited phases, BORTHWICK (4) and ROBBINS and BORTHWICK (12) with germination; COOLEY (5) with reserve cellulose of the seed. BLASBERG (3) has given some attention to anatomy. His work included growth habit, seedling development, root structure, and stem structure. SCHOLZ (13) has discussed morphology but has not given the detail of the development of the lateral roots and the root-stem transition. ARBER (1) has discussed the cladophylls and leaves. POESE (10) has dealt with the concentric and collateral vascular bundles of the rhizome and stem. BERNÁTSKY (2) has explained the development of the rhizome and the succeeding spears, and something of general structure. ZWEIGELT (17) has compared the members of the Asparagoideae on an anatomical basis.

The present study concerns itself with the details of the anatomy of the seedling, only parts of which have been reported previously.

Material and methods

In April, 1932, seeds of asparagus of the Mary Washington variety were obtained from Coker's Pedigreed Seed Company of Hartsville, South Carolina. To hasten germination, the seeds, after sterilization in 0.25 per cent Uspulun solution, were soaked in distilled water for three days in an incubator at approximately 30° C., the optimum

pre-germination time and temperature for soaking recommended by BORTHWICK (4). Although many seeds are injured by long soaking, especially at low and high temperatures, COUPIN (6) found asparagus seeds strongly resistant to immersion in water. Most of these seeds were planted out-of-doors in a loamy garden soil. To observe germination more closely, some were placed on moistened filter paper in sterilized petri dishes.

After germination began, seedlings were removed at intervals, killed and fixed chiefly in formalin-alcohol and in formalin-acetic-alcohol. Better fixations were obtained in the latter.

Following a suggestion of Dr. H. E. HAYWARD, it was found that heat would loosen the embryos from the soaked seeds so that they could be readily extracted. This made it possible to obtain the embryo from the hard endosperm more easily and with less injury than by dissection. Most of the embryos were obtained by heating in water to a temperature slightly below the boiling point, although no apparent injury resulted from heating the seeds to the boiling point.

The usual procedure for the paraffin method was followed, except that, to soften the lignified tissues, older parts of the plant were treated with 48 per cent hydrofluoric acid from one to five days before dehydration. A modification of the n-butyl alcohol method (16) was used in dehydrating these tissues.

Sections were cut 10 μ thick and stained in a modification of Flemming's triple stain. Detailed drawings were made by aid of a camera lucida and a microprojector.

Investigation

SEED AND EMBRYO

The ripe seed, covered with a black coat, the surface of which is finely rugose, is somewhat spherical or subglobose, rounded on the side away from the hilum, but flattened somewhat along one side of the hilum, producing a triangularly pyramidal effect on that side of the seed (fig. 1).

Most of the mature seed is composed of a hard cartilaginous endosperm with walls of hemicellulose (12, 5). The slender embryo, 3–4 mm. long, consisting chiefly of the haustorial cotyledon, is imbedded in the endosperm (fig. 1). There is generally a slight curve in the

distal portion of the cotyledon, although some very short embryos
are nearly straight. The embryo is blunter at the basal end, tapering
gradually to the distal end of the cotyledon. There is a longitudinal
slit in the cotyledon about 0.25 mm. in length, located just above the
cotyledonary plate (fig. 2). Through this slit, the margins of which
represent the edges of the cotyledonary sheath, the shoot will
emerge. The tissues of the cotyledon distal to the slit consist of an
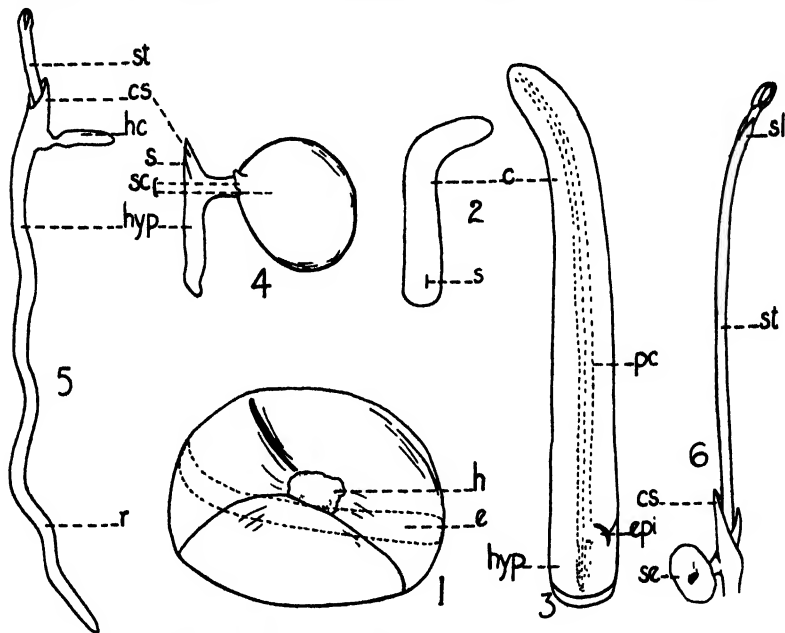
FIGS. 1–6.—Fig. 1, seed showing position of embryo. Fig. 2, embryo dissected from
seed. Fig. 3, diagram of longitudinal section of embryo. Fig. 4, young seedling showing
conelike development of cotyledon. Fig. 5, seedling with haustorial portion of cotyledon
dissected from seed. Fig. 6, part of older seedling (*h*, hilum; *e*, embryo; *s*, slit; *sc*, seed
coat; *hyp*, hypocotyl; *epi*, epicotyl; *c*, cotyledon; *pc*, procambial strand; *r*, root; *cs*,
cotyledonary sheath; *hc*, haustorial part of cotyledon; *sl*. scale leaf; *st*, stem; *se*, seed).

outer layer of papillate, densely protoplasmic, glandular cells; a re-
gion of six to ten rows of parenchymatous cells; usually three, some-
times four, procambial strands; and a central parenchyma. The epi-
cotyl is a hemispherical mass of embryonic tissue at the base of the
cotyledon within the small chamber below the slit (fig. 3). The short
hypocotyl has five or six xylem and as many phloem strands alter-

nately arranged, surrounding a pith. The xylem is generally not lignified. A single layer of pericyclic cells surrounded by the endodermis, ten to fifteen layers of cortical cells, and a single layer of epidermal cells complete the structure of the hypocotyl.

GERMINATION AND YOUNG SEEDLING

The tip of the hypocotyl lies very near the margin of the seed coat. Upon germination it emerges, tearing out a rounded disc of the seed coat, which, however, remains attached at one point. The basal portion of the cotyledon bearing the slit also emerges and enlarges so that it appears as a conelike structure which incloses the epicotyl (fig. 4). As development proceeds, there appears on the axis inside the cotyledonary sheath the first scale leaf. The next internode elongates appreciably so that the apical meristem and the leaf primordia it bears are brought well above the cotyledonary sheath and the first leaf (figs. 5, 6). The second and third leaves may exhibit a one-half phyllotaxy; the upper leaves are arranged in the two-fifths phyllotaxy. Growth of the parenchymatous cells of the haustorial portion of the cotyledon, by absorption of the surrounding endosperm, proceeds until the cotyledon almost fills the seed coats.

HYPOCOTYL AND PRIMARY ROOT

In the hypocotyl there are five or six protoxylem strands alternating with as many phloem strands. Metaxylem is differentiated toward the center surrounding a small pith and can be found in seedlings in the earliest stages of germination (fig. 7). Extensions from the lower portion of the hypocotyl develop into the primary root and possess the same arrangement of tissues. As the root matures the metaxylem differentiates at the central region until no parenchyma is left. Thus is formed the exarch radial protostele (fig. 8). The protoxylem consists of small thick-walled cells with spiral thickenings. The phloem is composed of sieve tubes, many small companion cells, and phloem parenchyma. The pericycle is limited to a single layer of large thin-walled cells. The endodermis has, at first, only small thickenings on the radial walls. Later the thickenings may extend to the inner tangential walls. The cortex consists of eight to twelve layers of cells, the outermost layer of which becomes suberized.

Development of the root axis is produced by two histogenic layers. This agrees with TREUB's findings (14) and represents a variation from type 2 of JANCZEWSKI (8). In the root tip in the Liliaceae TREUB found only two groups of distinctly separate initials: (1) the initials of the plerome, which are usually very few in number; and (2) a group of many cells outside of these, which serve as common initials for periblem, dermatogen, and cap (fig. 9). These latter ini-
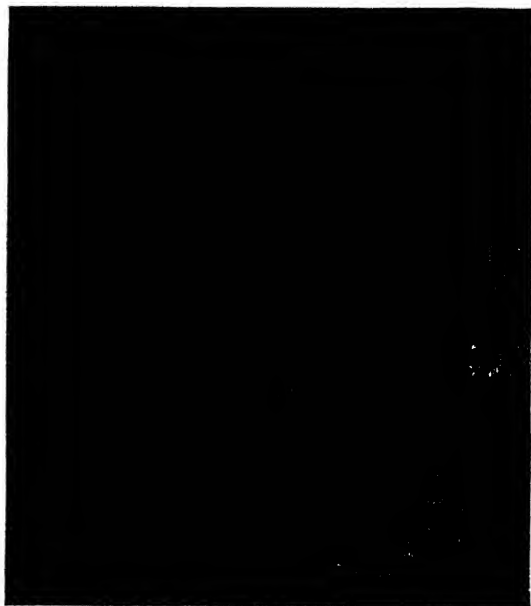
FIG. 7.—Transverse section of stelar region of primary root 1 mm. from root tip (*ph*, phloem; *mx*, metaxylem).

tials give the appearance of a general meristematic region from which new cells are derived with little regularity. Although rows of cells of the cortex and the epidermis may be traced into this region, the rows of cells of the cap are soon indistinguishable owing to irregular divisions of cells.

The mature primary root, less than 1 mm. in diameter and rarely more than 2 dm. in length, may persist throughout the first growing season, although its functions may largely be replaced by those of the large storage roots. The latter arise, apparently without any
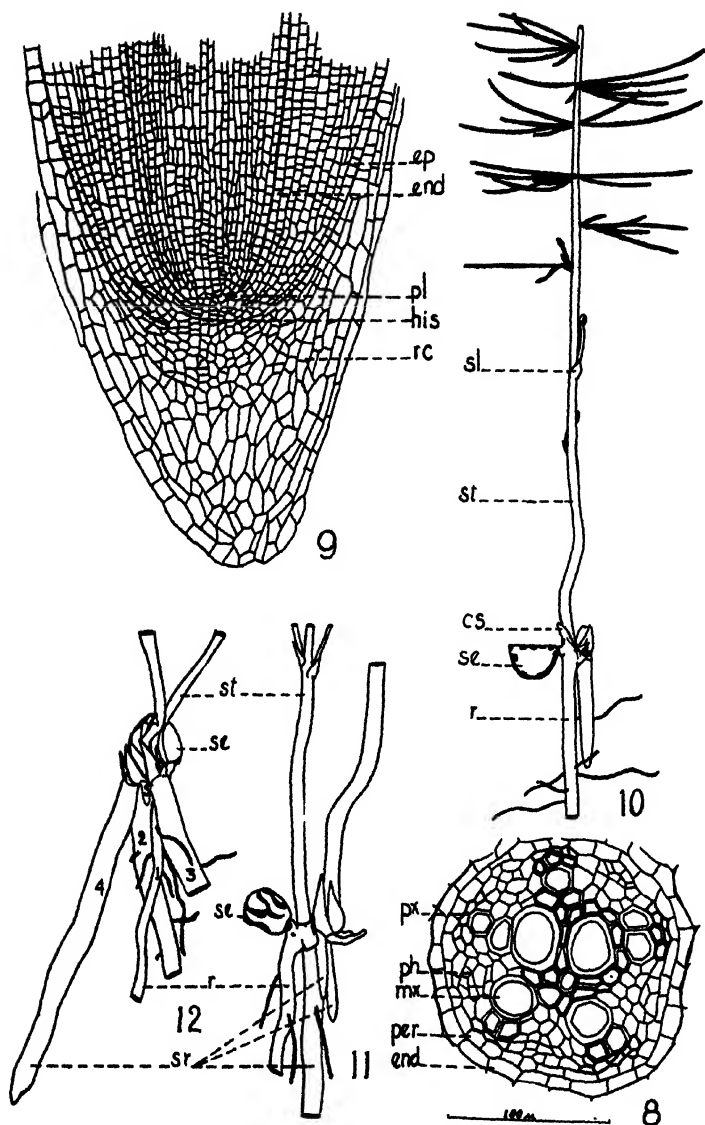
FIGS. 8–12.—Fig. 8, transverse section of exarch, radial protostele of primary root. Fig. 9, median longitudinal section of primary root. Figs. 10–12, portions of seedlings to show arrangements of parts (*ph*, phloem; *px*, protoxylem; *mx*, metaxylem; *per*, pericycle; *end*, endodermis; *ep*, epidermis; *rc*, root cap; *pl*, plerome; *his*, histogen for cortex, epidermis, and root cap; *se*, seed; *cs*, cotyledonary sheath; *st*, primary stem; *r*, primary root; *sl*, scale leaf; *sr*, storage roots).

order, from the lower side of the developing rhizome with such frequency and compactness that they become very crowded as the massive crown develops (figs. 10–12). These fleshy roots, with a diameter two to six times greater than the primary root, possess a pith, a large number of alternating xylem and phloem strands, and an extensive development of cortical tissue. The outer rows of cells of the cortex become thick-walled and suberized.

LATERAL ROOTS

Lateral roots originate in the pericyclic region of the primary root before the thickening of the walls of the large metaxylem vessels occurs. These branch roots are first noted as meristematic activity of the pericyclic cells usually directly opposite the protoxylem point. The first division of the pericycle is a tangential one (fig. 13). This is followed by other tangential divisions until four to six rows of cells are formed, when radial divisions also occur (figs. 14, 15). These divisions occur somewhat irregularly, but the cells tend to be laid down in rows parallel to the radial walls of the original pericyclic cells. Continued divisions of the pericycle produce a conical mass of meristematic tissue (figs. 16, 17). A secondary root halfway through the cortex of the primary root shows differentiation into two histogenic regions as found in the primary root (figs. 18, 19).

At about the time the first tangential divisions of the pericycle occur, the endodermal cells defining the tip of the root elongate and increase in size, keeping pace with the development of the pericycle (fig. 14). When radial divisions of the pericyclic cells occur, the endodermis over the developing root tip also undergoes radial divisions (fig. 15). Radial divisions of the endodermis are followed by tangential ones (figs. 16, 17), and by this means a considerable portion of the "temporary" root cap is derived from the endodermis (figs. 18, 19).

Lateral roots extend through the cortex partly by mechanical pushing and partly by digestion of the older cortical cells. The first is evidenced by the crushed cortical cells about the young root tips. In later stages there is a lack of accumulated débris about the tip of the secondary root, which may be considered as evidence that diges-
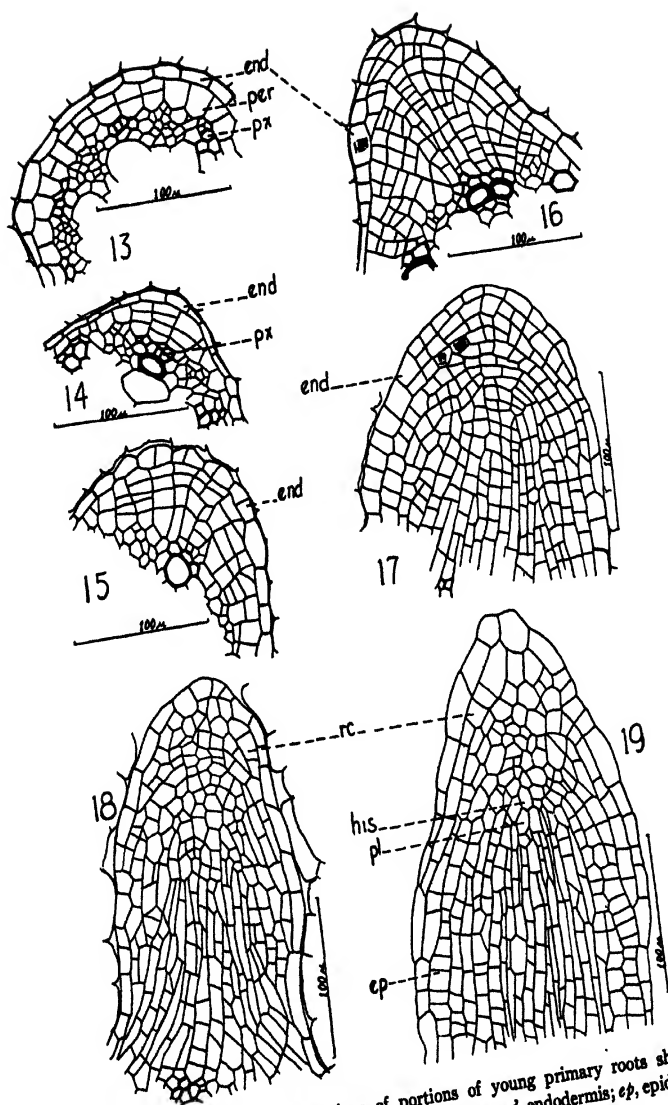
FIGS. 13-19.—Transverse sections of portions of young primary roots showing origin of lateral roots (*px*, primary xylem; *per*, pericycle; *end*, endodermis; *ep*, epidermis; *pl*, plerome; *his*, histogen for cortex, epidermis, and root cap; *rc*, root cap).

tion is important in the passage of secondary roots through the cortex.

These secondary roots continue development and extend beyond the limits of the primary roots as fibrous branches. They are usually triarch, with one large metaxylem vessel at the center.

TRANSITION REGION OF YOUNG EMBRYO

In a young embryo the cotyledon and the epicotyl are more or less in the same straight line, and in a longitudinal section the epicotyl appears as a somewhat lateral structure. Owing to the slight development of the epicotyl and the fact that very little conductive tissue is differentiated at this early stage within it, the transition must be determined largely as it takes place in the upper region of the hypocotyl and the cotyledon.

In a series of sections of a young embryo about 4 mm. long, in which the epicotyl is still only slightly developed, the transition from hypocotyl to cotyledon is evident. The orientation of the stelar tissues at successive levels from hypocotyl to cotyledon depicts the transition from the radial arrangement in the hypocotyl to the collateral arrangement in the cotyledon. A transverse section through the hypocotyl about 180 μ below the base of the epicotyl has the typical radial arrangement of the stelar structure. For convenience in following the orientation of tissues, the individual phloem and xylem strands have been given numbers and letters (fig. 20). All the xylem strands are not equally well differentiated, since the three (c, d, e) opposite the region which is directly below the apparently slightly lateral epicotyl are larger. A transverse section 80 μ higher includes the base of the cotyledonary sheath and the tissue just below the base of the epicotyl (fig. 21). The two weaker xylem strands (a, b) are not differentiated at this level, and xylem strands c, d, and e are slightly nearer the center. The phloem maintains its relative positions. At a still higher level, 100 μ above the first previous section, phloem strand 1 extends toward the base of the epicotyl. Extensions from the phloem strands 2 and 3, located right and left of 1, also are differentiated in the direction of the base of the epicotyl. The three stronger xylem strands (c, d, e) are nearer the center than in the previous section (fig. 22). A section 10 μ above the third level
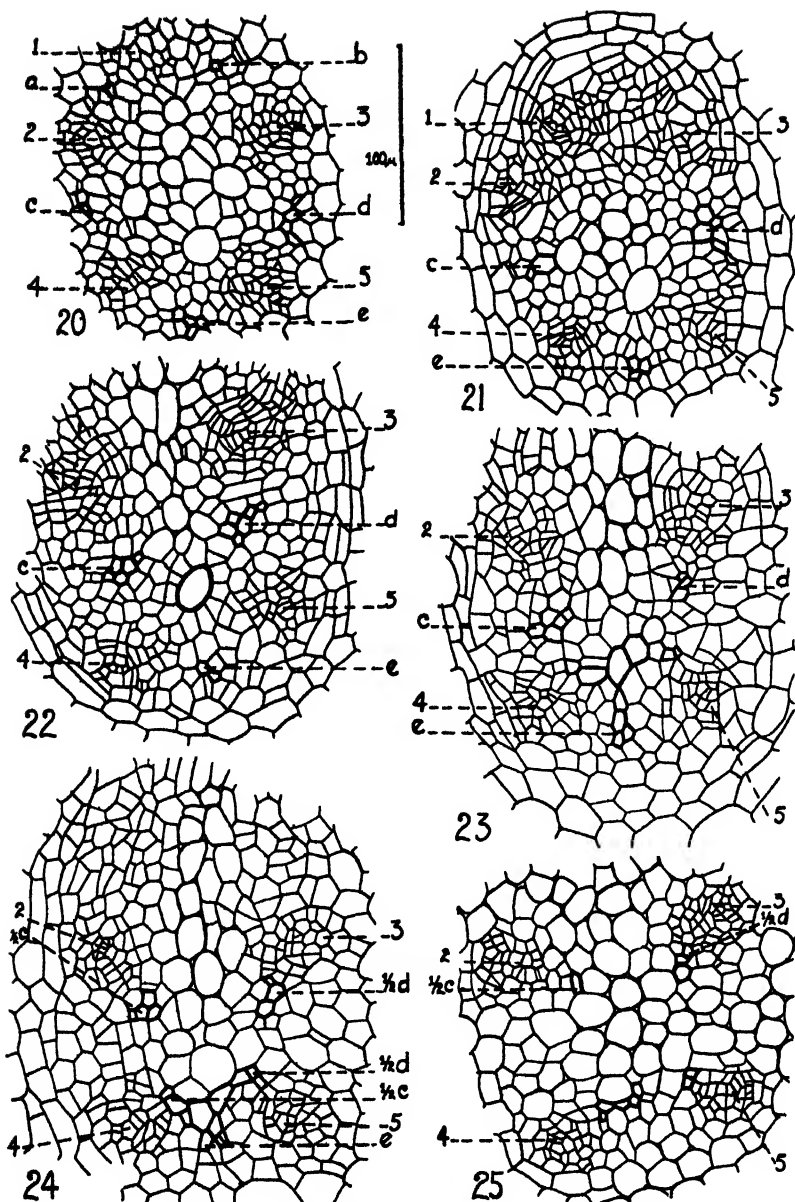
FIGS. 20–25.—Series of transverse sections of stele of young embryo to show transition; phloem strands numbered *1* to *5*, xylem strands lettered *a* to *e*.

has only four phloem strands, strand *1* having ended at the base of the epicotyl (fig. 23). The xylem strands are located nearer the center than in the section previously discussed; the metaxylem appears as a broken band, owing to lateral extensions right and left, and occupies a tangential position interior to the phloem strands. The fifth section, taken 10 μ above the previous one, shows the xylem located immediately interior to the phloem strands, toward the center of the cotyledon, the portions abutting on phloem strands *2* and *3* being extensions of the halves of the adjacent xylem strands *c* and *d*. The other halves of the xylem strands *c* and *d* are found abutting on phloem strands *4* and *5*. The lateral portions of the metaxylem of xylem strand *e* are found right and left, lying against phloem strands *4* and *5* (fig. 24). In a section 30 μ above this one all the bundles are collateral, the xylem, except for a small portion of strand *e*, being located against the phloem and nearer the center of the cotyledon (fig. 25). This portion of *e* at higher levels is found as an isolated strand of xylem.

In another young embryo there are five phloem and five xylem strands in the hypocotyl and only three vascular bundles in the cotyledon. In this instance, near the base of the epicotyl, two of the hypocotyledonary phloem strands and the three xylem strands nearest these are no longer present. The remaining three phloem strands are continuations of the three collateral bundles of the cotyledon. The xylem of these cotyledonary bundles is a continuation of the two xylem strands which alternated with these phloem strands in the hypocotyl.

Transition region of seedling

A seedling 47 mm. long was used as a type for study of transition in a young plant possessing a developed stem axis. The zone of transition is short and most of it was found to occur below the point of divergence of the cotyledon from the base of the epicotyl (fig. 26). In a transverse section of the hypocotyl, a little more than 1 mm. below the divergence of the haustorial portion of the cotyledon, the exarch radial arrangement is present (fig. 27).

A transverse section through the enlarged portion of the hypocotyl, 230 μ below the divergence of the cotyledon, has a localized meristematic region in the cortex (fig. 28). This is associated with
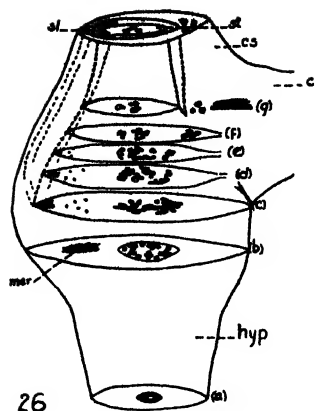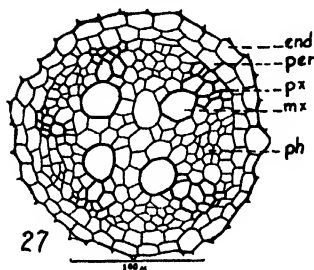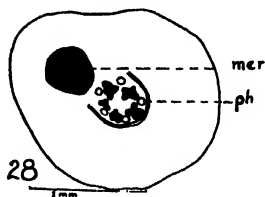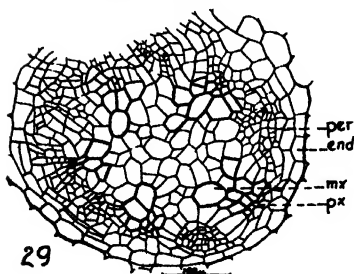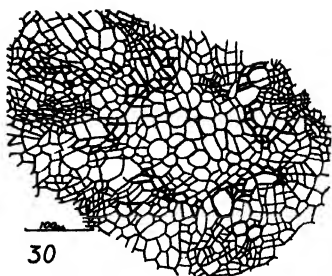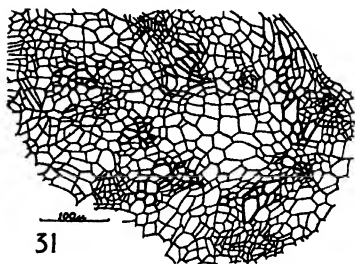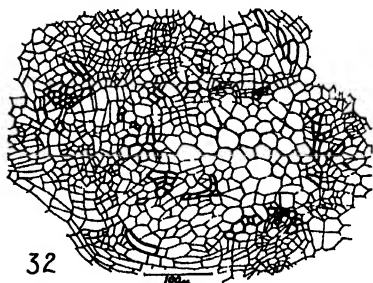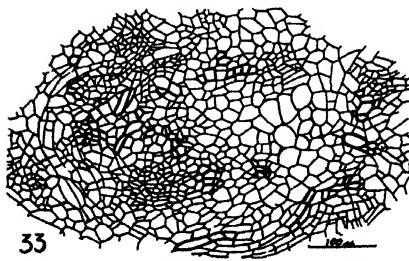
FIGS. 26–33.—Fig. 26, diagrammatic reconstruction of part of a seedling to show transition: sections at (a), (b), (c), (d), (e), (f), and (g) represent levels of figs. 27, 29, 30, 33, 36, 38, and 39. Fig. 27, transverse section of stelar portion of hypocotyl at level indicated by (a) of fig. 26. Fig. 28, diagram of transverse section of hypocotyl at level indicated by (b) on fig. 26. Figs. 29–33, part of a series of transverse sections, at successively higher levels, of transition region (cs, cotyledonary sheath; sl, scale leaf, c, cotyledon; hyp, hypocotyl; st, stem; mer, meristematic region).

the development of the bud which arises in the axil of the first scale leaf of the primary stem. The endodermis and pericycle are no longer evident on the side of the stele adjacent to this area, and the location of parts of the stele varies from the preceding section (fig. 29). The protoxylem points are a little nearer the center of the axis and the innermost metaxylem is situated laterally right and left and lies tangentially interior to the phloem, which maintains its relative positions.

In a section at the level of the divergence of the cotyledon there is a division of the conductive tissue into that part which is to continue into the stem and that part which is to continue into the cotyledon (fig. 30). The cotyledonary portion has four collateral bundles with some isolated xylem strands between these, the whole arranged in a circle. The cauline portion is identified by the location of some phloem and xylem outside of this circle. The arrangement is not clear, but the phloem lies external to the xylem. In the succeeding sections (figs. 31–37), 40, 110, 150, 170, 210, 270, and 310 μ above, there is considerable confusion in the arrangement of xylem and phloem tissues, since portions of many of the bundles appear in longitudinal rather than in transverse sections. This is largely due to the fact that the very short lower portion of the stem develops horizontally as a portion of the rhizome before growing upward as the aerial stem. This is not evident from the external structure because such a small portion of the stem is involved. Extensions of the vascular tissue toward the axillary bud may also account for some of the irregularity. Since the first scale leaf has no vascular tissue, it does not influence this confusion of tissues.

The haustorial portion of the cotyledon contains four vascular bundles. The cotyledonary sheath extending upward contains only three. One of these is larger and represents the upper undiverged portion of two bundles. The bundles of the haustorial portion connect at right angles with the bundles extending vertically from the cotyledonary sheath into the hypocotyl. The isolated xylem strands present in the lower portion of the stem axis (figs. 38, 39) apparently have no significance, since at higher levels they are identified as parts of the xylem of the nearest bundles. This also obtains in the cotyle-
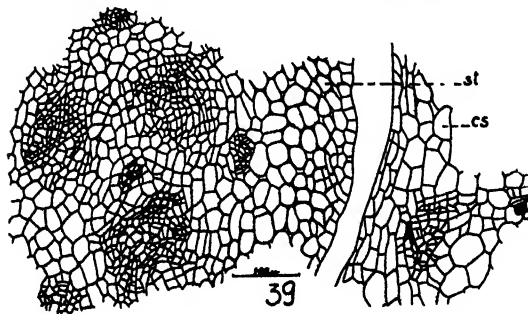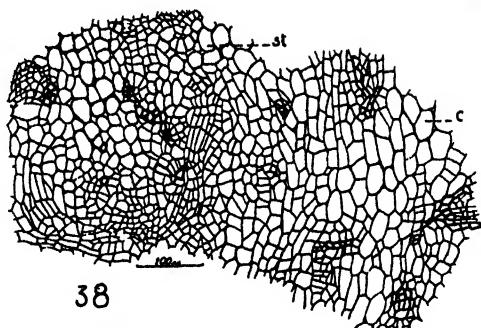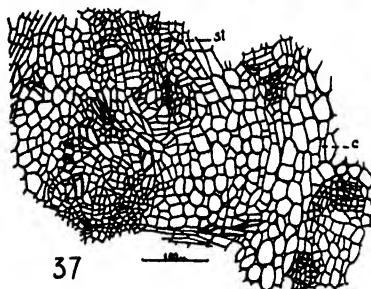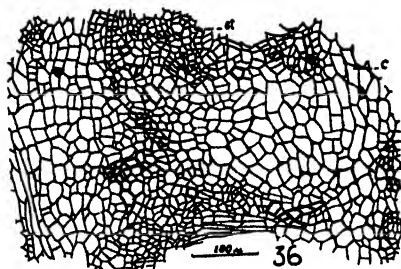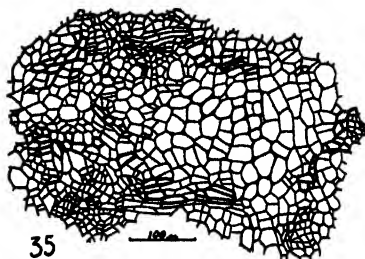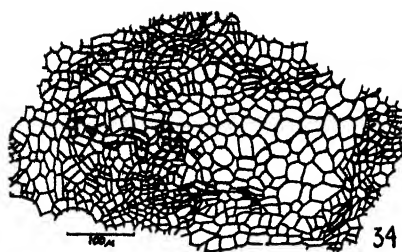
FIGS. 34-39.—Continuation of series of transverse sections at successively higher levels of transition region of a seedling.

dons where isolated xylem strands are found to be portions of the collateral bundles.

Other seedlings of similar age were examined for transition and were found to be similar to the one described. The transition may be considered a modification of type A of EAMES and MACDANIELS (7) or of type I of VAN TIEGHEM (15).

STEM

The lower portion of the main axis grows out horizontally and forms a small portion of the rhizome before the distal portion extends upward as an aerial shoot. The primary stem is slender and usually less than 2 dm. in height. The lower scale leaves (the first, second, and third) usually contain only branches in their axils. The upper scale leaves may bear both branches and cladophylls (fig. 10).

A transverse section of the primary stem reveals three zones. The first is the cutinized epidermis with stomata. The second is a varying number of rows of large cortical cells, the outer ones photosynthetic and with large intercellular spaces. The third is the stele, which may be divided into three parts: (a) a few rows of pericyclic cells which are strongly lignified in the lower, older portions of the stem and (b) the ground parenchyma located in the center between (c) the irregularly arranged vascular bundles (figs. 40–42). The larger bundles located near the center are the leaf traces, one large bundle associated with each leaf. These bundles are larger and more nearly centrally located in the vicinity of the node where they are diverged from the stele. At lower levels of the stem they are found near the periphery of the central parenchyma, in which region they may end blindly although usually they anastomose with traces of leaves at lower nodes. At the base of the stem there are anastomoses between the bundles of the stem and those of the rhizome. The small bundles which are found peripheral to the pericycle are portions of branch traces. In the upper, younger portions of the primary stem only one new branch trace is found at a node. At lower levels a second trace is differentiated and is present in the main stem at the node. Many anastomoses occur with the common bundles of the stem, but small portions of these branch traces, which do not immediately become part of the central stele, are found in the pericycle
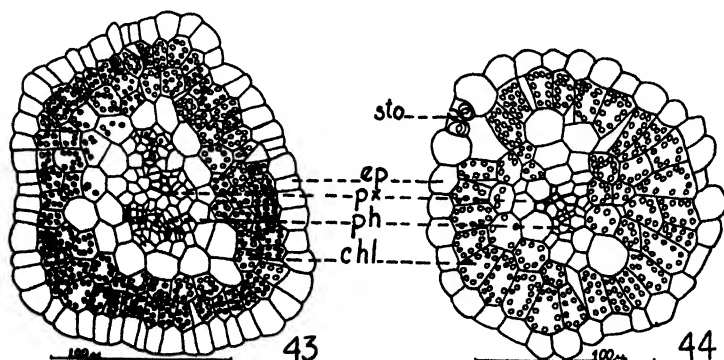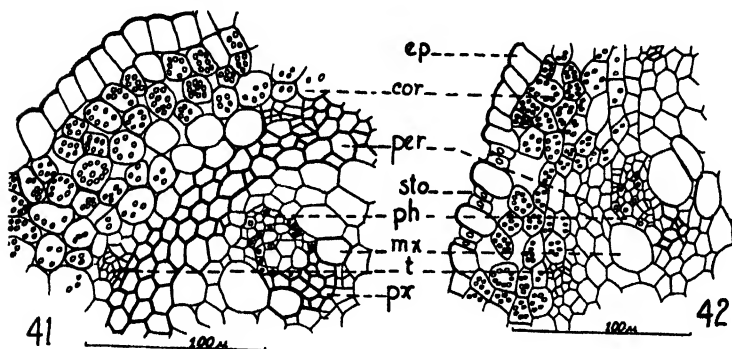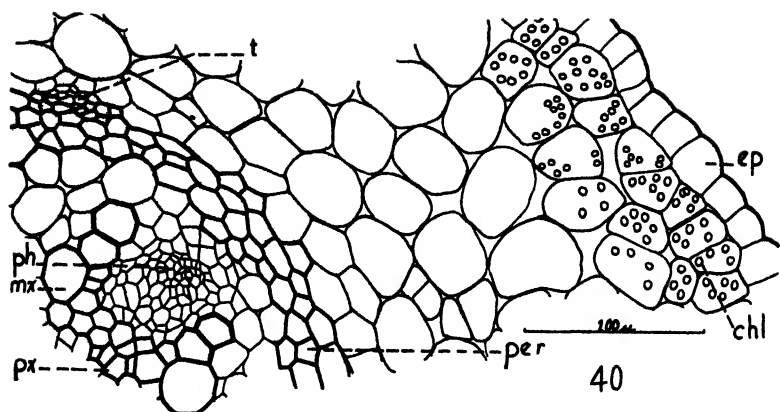
FIGS. 40–44.—Figs. 40–42, parts of transverse sections of lower, middle, and upper portions of primary stem. Figs. 43 and 44, transverse sections of cladophylls (*px*, primary xylem; *mx*, metaxylem; *per*, pericycle; *cor*, cortex; *ep*, epidermis; *sto*, stoma; *t*, branch trace; *chl*, photosynthetic area).

through an extent of several internodes before they pass through it and are found in the periphery of the central parenchyma. Here two or three may converge and anastomose with other traces.

The vascular bundles possess a V-shaped xylem arrangement with large reticulated vessels at the apices of the arms of the V and smaller spiral protoxylem vessels directed toward the center of the axis. The arms of the V inclose the phloem.

The bud, arising in the axil of the first scale leaf of the primary shoot, gives rise to a branch the short proximal portion of which forms the continuation of the rhizome, the distal portion the aerial shoot. The bud arising in the axil of the first scale leaf of this second shoot gives rise to the third shoot. As this process is continued, the bases of the aerial shoots form a compact rhizome made up of short internodes. Since the succeeding buds arise alternately right and left, the rhizome has two rows of spears in a close zigzag arrangement. Occasionally accessory buds develop, forming branches of the rhizome.

At the end of the growing season the aerial stems die, leaving scars on the older part of the rhizome. At the tip of the rhizome, however, buds for aerial stems of the next season are already present.

CLADOPHYLLS

The cladophylls are essentially stem structures (fig. 10). They are terete or polygonal in transverse section and have no morphological dorsiventrality. There is a cuticularized epidermis with slightly depressed stomata, the guard cells of which are considerably smaller than the vertically elongated epidermal cells. This differs from ZWEIGELT's description (17) which includes accessory cells associated with the stomatal apparatus. Beneath the epidermis there are two to three rows of palisade cells crowded with chloroplasts. These are followed by the ground parenchyma and from one to four collateral vascular bundles. Two was found as the common number in young cladophylls (fig. 43). A section near the apex of one has a single vascular bundle (fig. 44). In older cladophylls lignified sheaths are developed about the vascular bundles. The vascular connection of the cladophylls with the primary stem is made through an anastomosis of the small bundles of the cladophylls with the large bundle of the scale leaf.

Scale leaves

The sessile scale leaves are approximately triangular in shape, but possess a small, median, ventral prolongation. Usually no vascular tissue or very little is present in the first scale leaf. In the others there are usually four main bundles, two of which extend laterally at the base, and two of unequal size which extend upward in the central region. Small vascular bundles may extend upward from the two lateral bundles. All of these bundles converge into one at the base of the leaf, and vascular connection with the stem is made through this bundle.

Summary

1. The structure of the embryo and the development of the seedling of *Asparagus officinalis* L. are described.

2. The primary root is usually pentarch or hexarch. In its growing point there are two histogens, the inner giving rise to the stele and the outer to the cortex, epidermis, and root cap.

3. Lateral roots are initiated by tangential divisions of cells of the pericycle. Subsequent tangential and radial divisions continue the development.

4. In the development of lateral roots the endodermis becomes active and divides by tangential and radial divisions to form a considerable portion of the "temporary" root cap.

5. Transition from an exarch to an endarch condition of the xylem occurs in the hypocotyl and the bases of the cotyledon and the epicotyl. The transition is complicated by the development of both a part of the rhizome and the first aerial branch from the epicotyl.

6. The rhizome is formed from horizontal outgrowths of the base of the epicotyl and the bases of successive aerial stems.

7. Successive aerial branches arise from buds in the axils of the first scale leaves of the preceding aerial branches.

8. The leaves are scale leaves with one large trace which extends toward the center of the central parenchyma of the stem. From this region the trace gradually diverges outward until, at lower levels of the stem, it is found near the periphery of the central parenchyma. Here it may end blindly but usually anastomoses with traces of leaves of lower nodes.

9. The young branch, arising in the axil of a scale leaf, has small

traces which anastomose with the common bundles of the stem. Small branches of these traces remain in the outer pericycle, through several internodes of the main stem, before they pass through it and come to lie in the peripheral region of the central parenchyma, where they anastomose with other bundles.

10. The cladophylls are essentially stem structures. They arise in clusters in the axils of the scale leaves.

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LITERATURE CITED

1. ARBER, AGNES, *Myrsiphyllum* and *Asparagus:* a morphological study. Ann. Bot. 38:635–659. 1924.
2. BERNÁTSKY, J., Mit Beiträgen von Kirchner: Asparagoideae. Kirchner, O. von, Loew, E., und Schröter, C.: Lebensgeschichte der Blütenpflanzen Mitteleuropas. 1: Abt. 3. 615–686. 1914.
3. BLASBERG, C. H., Phases of the anatomy of asparagus. BOT. GAZ. 94:206–214. 1932.
4. BORTHWICK, H. A., Factors influencing the rate of germination of the seed of *Asparagus officinalis*. California Agr. Exp. Sta. Tech. Paper 18. 1925.
5. COOLEY, G. E., On the reserve cellulose of the seeds of Liliaceae and of some related orders. Boston Soc. Nat. Hist. Mem. 5:1–29. 1895.
6. COUPIN, HENRI, Sur la résistance des graines à l'immersion dans l'eau. Compt. Rend. Acad. Sci. Paris 126:1365–1368. 1898.
7. EAMES, A. J., and MACDANIELS, L. H., An introduction to plant anatomy. McGraw-Hill, New York. 1925.
8. JANCZEWSKI, EDUARD, Recherches sur l'accroissement terminal des racines dans les phanérogames. Ann. Sci. Nat. Bot. Sér. 5. 20:162–201. 1874.
9. JONES, H. A., and ROSA, J. T., Truck crop plants. McGraw-Hill, New York. 1928.
10. POESE, OSWALD, Über die Leitungsbahnen einiger Liliaceen. Beilage zum Programm der Luisenschule zu Berlin. Ostern. 1912.
11. ROBBINS, W. W., The botany of crop plants. Blakiston's, Philadelphia. 1924.
12. ROBBINS, W. W., and BORTHWICK, H. A., Development of the seed of *Asparagus officinalis*. BOT. GAZ. 80:426–438. 1925.

13. Scholz, Eduard, Entwicklungsgeschichte und Anatomie von *Asparagus officinalis* L. Festschr. zum 50. Jahres-Bericht der Schottenfelder K. K. Staats-Realschule im VII Bez. Wien. pp. 137–151. 1900–1901.

14. Treub, M., Le méristème primitif de la racine dans les monocotylédones. Musée Botan. Leide. Vol. II. 1875.

15. van Tieghem, P. E. L., Traité de botanique. Paris. Vol. I. 1891.

16. Zirkle, Conway, The use of n-butyl alcohol in dehydrating woody tissue for paraffin embedding. Science 71:103–104. 1930.

17. Zweigelt, E. F., Vergleichende Anatomie der Asparagoideae, Ophiopogonoideae, Aletroidreae, Luzuriagoideae und Similiacoideae nebst Bemerkungen über die Beziehungen zwischen Ophiopogonoideae und Dracaenoideae. Denkschr. K. Akad. Wissensch. Math. Naturw. Kl. Wien 88:397–476. 1912.

A METHOD OF MEASURING RESPIRATION AND CARBON FIXATION OF PLANTS UNDER CONTROLLED ENVIRONMENTAL CONDITIONS[1]

JOHN W. MITCHELL

(WITH FIVE FIGURES)

Introduction

In various studies of the rates of respiration and carbon fixation, many methods have been developed to control as nearly as possible the several environmental factors involved. As yet none has proved satisfactory. It is the purpose to give here a statement of the methods and a brief description of the apparatus used in making such studies when the plants used were inclosed in glass chambers and exposed to a moving air stream. Temperature, light, humidity, CO_2 supply, and the rate of air circulation were controlled in such a way as to maintain nearly constant conditions inside such chambers.

Temperature was readily controlled by means of an insulated room having properly regulated thermostats, cooling system, and heaters. A means of controlling the humidity inside the chambers was not necessary since it remained nearly constant during the period over which measurements were taken. Because of the wide fluctuations in intensity and quality of natural daylight, it was necessary to employ some other source of illumination which could be precisely regulated. An arc lamp was finally selected as the most suitable source of artificial illumination. It was found desirable to enrich the air stream supplied the plants under test by the addition of a known amount of CO_2. The air around the leaves under study constantly recirculated. This was necessary to make sure that the leaves or plants were constantly bathed by a more nearly uniform concentration of CO_2 and also to avoid the use of quantities of gas supplied in excess of the amount that could be readily and accurately measured.

Details of apparatus used

TEMPERATURE.—An insulated room, provided with coils connected to an ammonia compressor and temperature control unit, was used. By means of this apparatus it was possible to maintain a room temperature of 69°–71° F. during periods of illumination, and 64°–66° F. during periods of darkness.

When the light was on, during the measurements of rates of carbon fixation, the temperature of the air inside the leaf chamber rose about 4° C. above that of the room. In order to measure the temperature of the leaves themselves, a potentiometer and thermocouples made from fine constantine and copper wire, insolated with a thin coating of paraffin, were employed. Readings were made with the couples against the lower side of the leaves as well as threaded through them. The readings were approximately the same regardless of the position of the couples. During illumination the temperature of the leaves increased approximately 2° C. above the surrounding air, whether inclosed in the chambers or not. MILLER (3) found similar differences between the temperature of leaves and the surrounding air under natural conditions.

HUMIDITY.—The relative humidity of the air in the room ranged between 60 and 75 per cent, while that of the air inside the chambers ranged from 50 to 80 per cent, depending upon the size and type of leaf used. When large succulent leaves were used, the relative humidity in the chamber usually ranged from 65 to 70 per cent and when small leaves were inclosed the range was from 50 to 65 per cent.

ILLUMINATION.—In the selection of a source of artificial light under which various plants, particularly the tomato, could be suitably grown, several qualities and intensities of light were tested. Tungsten filament electric lamps varying in output from 500 to 2000 watts were first tried. The quality of light from such a source was altered by using gelatin filters of various types, and also by operating 105, 108, and 110 volt lamps at 112 volts. When supplemented by no other source of light, plants which had previously been grown to some size before being placed under experimental conditions showed some variation in their color but none of them accumulated an

appreciable carbohydrate reserve, and all showed symptoms of etiolation within seven days. The use of a considerable number of such lamps in a room means the production of a great quantity of heat. As a result it was not possible under local conditions to control room temperatures readily. The use of tungsten lamps then was abandoned and an Eveready carbon arc lamp substituted. The arc of this lamp was completely inclosed by glass filters, held in place by removable metal frames. By means of an exhaust fan a constant supply of fresh air was drawn from outside the experimental room to the bottom of the glass inclosed chamber about the arc, through this chamber, out at its top, and exhausted outside the building. In this way it was possible to remove most of the heat and any gases which might influence the development of the plants.

This arc lamp operated on a current of 60 amperes with 50 volts at the arc. Plants were placed in a circle around the lamp at a distance of 30 inches, the light striking the leaves at an angle of 45°. Carbons known as the "sunshine" type produced a suitable quality of light. Energy radiation from the arc produced by such carbons consisted qualitatively of a spectrum which was practically as continuous as that of sunlight. A spectrogram shows that the spectral lines extend from the infrared through the visible and ultraviolet to approximately 2800 A.u. A comparison of the energy radiation curves of noon-day summer sunlight and artificial light from sunshine carbons (fig. 1) shows that sunlight contains more red, but less blue. The quality of natural sunlight itself is by no means constant and measurements vary greatly, depending upon the time and place at which they are made. On the basis of an assumed average quality of natural sunlight, the quality of light produced by sunshine carbons gives a closer approximation than does the light produced by any of the other types of carbons or by the tungsten filament lamps. To be of use in the present experiments, however, it was found that a filter was necessary to remove the excess amount of ultraviolet. With a filter of Corex D glass, which transmits most wave lengths from the visible down to about 2500 A.u., tomato and bean plants were killed upon being exposed to this light for a period of 2–5 hours. The use of pyrex glass filters, which removed rays shorter than 2700 A.u., resulted in injury to plants in 1–2 weeks. Single strength window

glass, which removed most rays shorter than 3200 A.u., was finally selected, since no injury resulted from its use.

As previously indicated, it is difficult to make a quantitative comparison of artificial light and natural sunshine, owing to the great range of variability in the intensity of natural light. A rough comparison of intensities of the light from screened arc and natural daylight on cloudy and clear days was made. Measurements made with
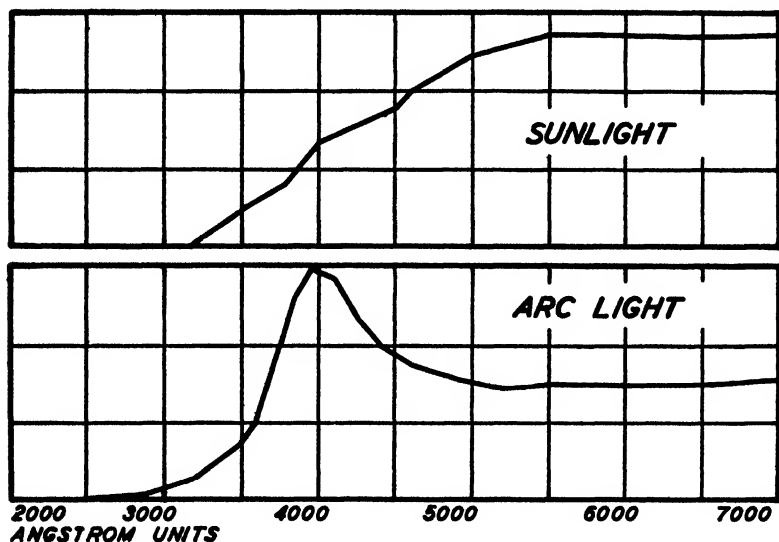
FIG. 1.—Comparison of energy radiation of noon-day summer sunlight and arc light from "sunshine" carbons. In the graph illustrating sunlight each square represents 2500 micro-watts of radiant flux per square cm. Each square in the graph illustrating arc light represents 250 micro-watts per square cm. at 1 meter distance from the arc. By courtesy of the National Carbon Company.

a thermopyle, MacBeth illuminometer, and Weston light meter showed that the intensity of total or visible radiant energy of the arc at a distance of 3 feet was very much less than that of direct sunlight at noon on a clear day in April. However, the intensity of total or visible radiant energy from the arc light exceeded that of natural light during a day in the same month when the sky was completely overcast. In some of the first experiments performed, it was noticed that there was a 15–20 per cent increase in the total radiant energy produced by any given set of carbons during the last half of their

burning time. This increase in intensity was possibly due to the infrared radiations produced by metallic parts of the lamp which moved nearer to the arc and increased in temperature as the carbons were consumed. Such an increase in intensity was entirely eliminated by passing the light from the arc through a water filter. The continued use of such a water filter was not necessary, however, as experiments have shown that a 20 per cent increase in intensity of infrared radiation, during the life of a set of carbons, has no noticeable effect on the rate of carbon fixation by the plants studied.
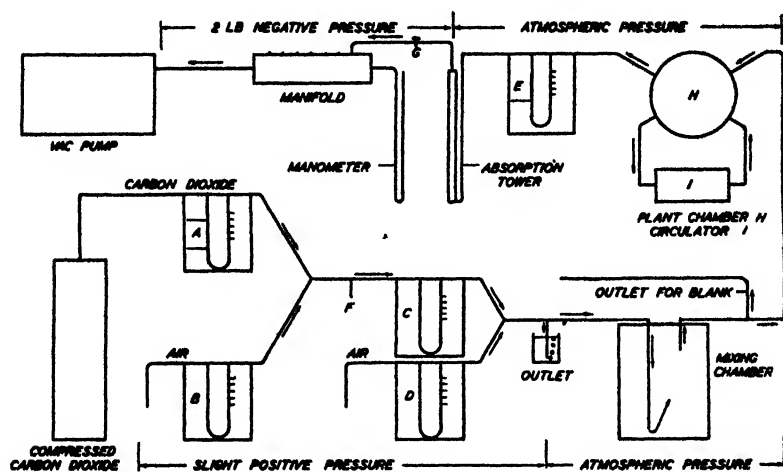
Fig. 2.—Arrangement of apparatus used to measure rates of respiration and carbon fixation.

MOVEMENT OF ENRICHED AIR THROUGH THE SYSTEM.—The volume of gas which was supplied to the leaf chambers was derived from a tank of compressed air, the volume available at any particular time being slightly in excess of the amount subsequently to be drawn over the leaves. A flowmeter (fig. 2D) of the type described by WILSON and GEORGI (7) was used to indicate the flow. The CO, which was used to enrich the main air stream was derived from tanks of the compressed gas. From such a tank the gas was first fed through a flowmeter (fig. 2A) and mixed with air coming from the compressed air tank at a known rate as indicated by the flowmeter (fig. 2B). In this manner suitable quantities of a mixture of air and CO, in any

desired proportions could be had. In practice the volume available was always slightly in excess of the amount finally to be drawn over the leaves in the chambers. Such an excess was allowed to escape through an outlet immersed in oil, thus preventing any other gases from entering the system. Up to this point the gases were under slight positive pressure. From now on, however, the gases traveled through the remainder of the system because of a negative pressure set up by the use of a vacuum pump at the extreme end of the line, the degree of negative pressure at any point in the system being regulated by needle valves and determined by flowmeters at various points as described later. Thus beyond the escape valve just mentioned the air enriched with CO_2 passed, at virtually atmospheric pressure, through copper tubing to the leaf chamber, where it was recirculated several times by means of a special pump connected with the chamber, before passing on out of the chamber into a tube which fed through a flowmeter to an absorption tower, kept at constant temperature by immersion in a controlled water bath. The rate of flow into the tower was controlled by a needle valve (fig. 2G) and the flow gauged by a meter (fig. 2E). After passage through the absorption tower, the remaining gases passed through copper tubing into a manifold where a slightly greater negative pressure was maintained and controlled by a needle valve and indicated by a manometer. Beyond the needle valve a tube led directly to the vacuum pump.

Seven sets of the apparatus described were assembled as a unit and operated by a single vacuum pump. Thus as many as seven air streams could be drawn from a common source simultaneously, six of them across leaves or plants, the seventh used as a check to determine the amount of CO_2 in the enriched air supply.

LEAF AND PLANT CHAMBERS.—Two types of chambers were devised, one of which was used to inclose an attached leaf, the other being used to inclose an entire plant. The frame of the first type was constructed from four brass rings of the relative dimensions shown in figure 3 (A, B, C, D). Corex D glass was used in the side of the chamber nearest the light source (fig. 3E), while single strength window glass was used in the side away from the light (F). Rubber gaskets were inserted on each side of both glasses and shellac applied

to prevent air leakage. A removable metal frame bearing fine wires was inserted in each half of the chamber for the purpose of holding the leaf away from the glass. The excess space outside of these frames was filled with plaster of Paris to decrease the volume within the chamber. Several applications of a commercial lacquer were used to reduce the porosity of the plaster and thus prevent the adsorption
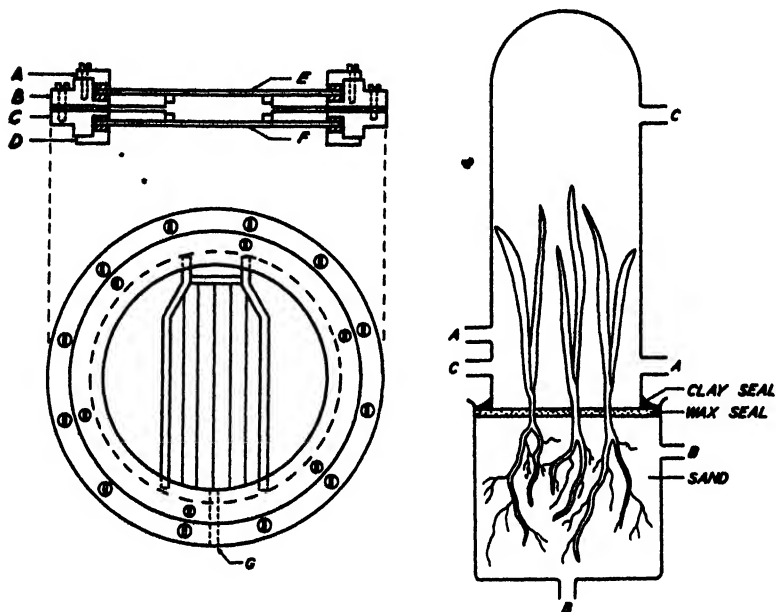
Figs. 3, 4.—Fig. 3 (left), diagram of leaf chamber. Fig. 4 (right), diagram of plant chamber; air stream connected to outlets AA, another to BB, and a circulator to outlets CC.

of appreciable quantities of gas. The parts of the chamber were fastened together with screws and the union between the upper and lower halves made air tight by means of a rubber gasket. A hole was drilled at the union of the two halves of the chamber through which a leaf petiole could be inserted (fig. $3G$). Four pieces of pipe, extending through the metal walls, made it possible to recirculate air through the chamber. The chamber was finally mounted on an adjustable stand as shown in figure $5A$.

The second type of chamber consisted of an upper part inclosing

the stems and leaves and a lower part inclosing the roots of the plants (fig. 4). The lower part of the chamber consisted of a 1.5 liter beaker provided with two outlets through which air could be drawn. One of these outlets was at about the center of the bottom, and the other about three-fourths of the distance up the side. The beaker was filled with quartz sand in which plants were grown until they attained the desired height. A paraffin, vaseline, and mineral oil
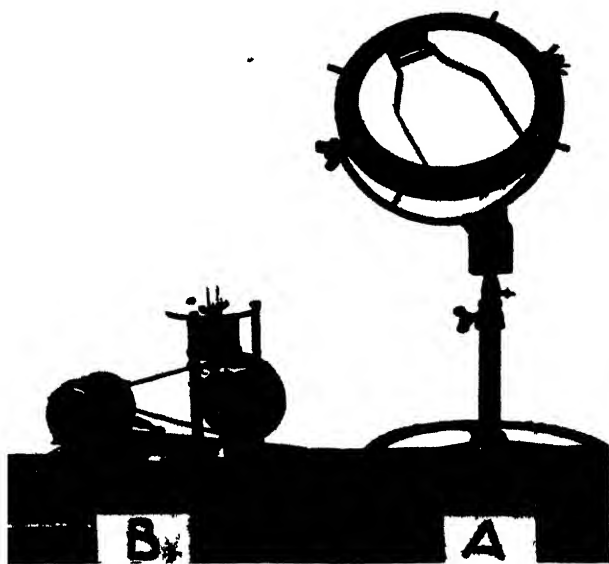
FIG. 5 —B, circulatory pump; A, leaf chamber

mixture, having a melting point of 38° C., was heated to a temperature of 41° C., then poured over the surface of the sand and allowed to flow around the stems of the plants and solidify. Thus the lower portion of the chamber was effectively sealed, so that gases could neither enter nor leave except through the openings already mentioned.

The upper part of the chamber, which was constructed of large pyrex tubing and provided with four openings, was placed over the portion of the plants extending above the paraffin seal. An air tight connection was made between the upper chamber and the paraffin seal by means of a mixture of clay and castor oil.

Through two of the openings gas entered and left the chamber at the rate of 15 liters an hour. This rate of movement over the leaf surface, however, was not rapid enough for the most effective absorption of CO_2. Consequently tubes leading to a circulation pump were attached to the other two openings so that gases in the chamber were constantly agitated and moved over the leaf surfaces at a rate equivalent to a complete flushing of the chambers 200 times per hour, or a volume of gas equivalent to 400 liters.

CIRCULATORY PUMPS.—A pump was devised to circulate the air inside the chamber. Its usefulness depended on the fact that it could be made air tight and could circulate the desired volume of air for a period of several weeks without attention.

In the construction of these pumps a length of metal bellows 3 inches in diameter and 2.5 inches long was attached to a circular brass plate held in a horizontal position by upright rods (fig. 5B). Inlet and outlet valves were inserted in the plate. Another circular brass plate, somewhat smaller in diameter, was attached to the lower end of the bellows and connected to an excenter on a drive shaft by means of a connecting rod and suitable bearing. The mechanism was operated by a small motor.

A pump of this type delivered from 350 to 500 liters of air per hour. When attached to a leaf chamber having a volume of 500 cc., the volume of gas in the chamber was flushed at the rate of approximately 800 times per hour. The minimum volume of gas circulated over a leaf having an area of 100 square cm. was therefore between 30 and 40 liters per square cm. of leaf surface per hour. This rate of circulation was greatly in excess of that required for the maximum rate of carbon fixation by leaves, as shown by the work of HEINICKE and HOFFMAN (2).

ESTIMATION OF CO_2.—The conductivity method was used for the estimation of $\dot{C}O_2$ in an air stream to which leaves had been exposed. The absorption towers were similar in construction to those described by THOMAS (6).

These towers were charged with 50 cc. of LiOH solution containing 0.5 to 1.0 per cent of butyl alcohol. Air moving at a rate of 15 liters per hour through the absorbent was dispersed as fine bubbles not more than 2 mm. in diameter.

The procedure used in the standardization of an absorption tower and alkali absorbent solution was as follows: the tower was filled with 50 cc. of absorbent, placed in a water bath, and brought to the temperature to be used. It was then connected to an air stream and an amount of CO_2 was collected. The electrical resistance of the solution was then determined with a wheatstone bridge. The solution was removed from the tower and an aliquot titrated with HCl as described by FRIEDEMANN and KENDALL (1). This process was repeated several times, absorbing larger amounts of CO_2 for each determination. A curve was finally drawn from the data collected, which indicated the resistance of the alkali solution in the tower used in terms of milligrams of CO_2. Towers and solutions standardized in this way have been used over an extended period without a noticeable change after standardization.

Dependability of results

It has been shown by SPOEHR and McGEE (5) and NEWTON (4) that very small amounts of CO_2 may be accurately measured by the conductivity method. It should be noted, however, that the efficiency of dilute alkaline solutions used to absorb CO_2 decreases as the hydroxide is changed to bicarbonate and carbonate, through the addition of CO_2. As much as 150 mg. of CO_2 could be absorbed by 50 cc. of 0.25 N LiOH under the conditions used, without appreciably limiting the absorbing capacity of the alkali.

In the use of an apparatus which measures simultaneously the rates of respiration or carbon fixation of several leaves, it is important to know that the same degree of accuracy is exhibited by the several separate units of the system. Two air streams, drawn simultaneously from a common air source, were analyzed to determine the accuracy of the method described in this study. The movement of air in the two lines was adjusted to an equal velocity. A total of 95 liters of air was drawn through each system and the amount of CO_2 collected from each air stream was determined at the end of every period required for the passage of 19 liters. The data thus obtained showed the difference between the amounts of CO_2 collected from the two air streams to be less than 5 per cent. Analyses were also made, using six separate units of the system simulta-

neously, the results showing that 0.61, 0.59, 0.58, 0.59, 0.58, and 0.61 mg. of CO_2 per liter were collected from air drawn through the six respective units. Again an error of about 5 per cent resulted.

Tomato, bean, primrose, geranium, and cineraria plants produce healthy vegetative growth under the environmental conditions described. A comparison of the approximate rates of CO_2 assimilation

TABLE I

COMPARISON OF APPROXIMATE RATES OF CARBON FIXATION BY VARIOUS PLANTS. THE CONCENTRATION OF CO_2 IS THAT WITHIN THE RANGE OF VARIABILITY FOUND UNDER NATURAL CONDITIONS

DATE	PLANT	UPTAKE MG. CO_2 PER HOUR PER 100 SQ. CM. LEAF SURFACE	LIGHT SOURCE	INVESTIGATOR
1884........	Sunflower	18 0	Sunlight	Sachs
1917........	Pumpkin	18 0	Sunlight	Miller
1917..... .	Cow pea	18 5	Sunlight	Miller
1917........	Soy bean	8 0	Sunlight	Miller
1920.	Sugar cane	3 5	Sunlight	McLean
1933... ...	Apple	15 0	Sunlight	Heinicke and Hoffman
1934..... ..	Geranium	16 5	Arc light	Mitchell
1934.. . .	Tomato	17 0	Arc light	Mitchell
1934........	Primrose	13 5	Arc light	Mitchell
1934...	Cineraria	13 5	Arc light	Mitchell
1934... .. .	Wax bean	13.0	Arc light	Mitchell

by leaves of these plants under the conditions described and the results obtained by other investigators using other methods is given in table I.

Summary

1. An apparatus is described with which the hourly rates of carbon fixation or respiration of attached leaves can be accurately determined. Descriptions of a new type of circulatory pump and two new types of leaf chamber are given.

2. Artificially controlled environmental conditions are described in which several species of plants have gained in dry weight over a period up to four weeks.

3. Of the sources tested, an Eveready carbon arc lamp, inclosed by a housing of window glass, was found to be the most suitable for maintaining plants under conditions of artificial light.

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LITERATURE CITED

1. FRIEDEMANN, T. E., and KENDALL, A. I., The determination of carbon and carbon dioxide. Jour. Biol. Chem. 82:45–55. 1929.
2. HEINICKE, A. J., and HOFFMAN, M. B., The rate of photosynthesis of apple leaves under natural conditions. I. Cornell Univ. Agr. Exp. Sta. Bull. 577. 1933.
3. MILLER, E. C., Plant physiology. McGraw-Hill, New York. 1931.
4. NEWTON, R. G., An improved electrical conductivity method for the estimation of carbon dioxide and other reactive gases. Ann. Bot. 49:381–398. 1935.
5. SPOEHR, H. A., and McGEE, J. M., Studies in respiration and photosynthesis. Carnegie Inst. Washington. 1923.
6. THOMAS, M. D., Precise automatic apparatus for continuous determination of carbon dioxide in air. Ind. Eng. Chem. Anal. Ed. 5:193–198. 1933.
7. WILSON, P. W., and GEORGI, C. E., Methods for controlling the environment of greenhouse plants. BOT. GAZ. 94:346–363. 1932.

CORRELATION OF SHAPE OF FRUITS, COTYLEDONS, AND SEEDS IN MELONS[1]

LESLIE M. WEETMAN

(WITH EIGHT FIGURES)

In connection with the breeding of wilt-resistant watermelons (*Citrullus vulgaris* Schrad.), it was observed that plants with ovate or nearly circular cotyledons commonly bore spherical fruits and that plants with elongated cotyledons bore elongated fruits. This was particularly noticeable in the new wilt-resistant Iowa Belle variety, which originated from a chance hybrid heterozygous for fruit shape. The apparent correlation between shape of cotyledons and shape of fruits suggested that the shape of the fruits a plant will bear might be foretold by observing the shape of the cotyledons. This would greatly expedite the selection of strains homozygous for round or long fruits. With this idea in view, a study was started to determine more definitely the relationship between shape of cotyledons and shape of fruits in the Iowa Belle and in a number of other varieties. It was also desirable to determine whether there is a correlation in shape between seeds and cotyledons and between seeds and fruits.

HUTCHINS (6) has reported that the cotyledons, fruits, and seeds of cucumbers are closely correlated in shape. He calculated correlation coefficients from the mean shape indices of a number of varieties and from individual indices in an F_2 population of 300 plants. Length and shape of cotyledons and seeds were more influenced by the female than by the male parent in hybrids. With regard to matrocliny in the cotyledons of hybrids, FOCKE (2) cited the observations of CASPARY that reciprocal hybrids of *Nymphaea rubra* and *N. dentata* differed in the shape of the cotyledons. The cotyledons of the hybrid resembled those of the female parent in each case. GROTH (4) found in tomatoes that both size and shape of F_1 cotyledons were affected by the size and possibly by the shape of the seed

of the female parent. HILL (5) reported that reciprocal F_1 hybrids of species of *Digitalis* differed considerably in the form and size of their cotyledons, and that they always resembled the female or seed parent in these respects, although this matrocliny was not complete. He thought the explanation lay in the influence of the maternal seed coats upon the developing embryos. LUBBOCK (7) described seedlings of a number of species of the Cucurbitaceae and attributed the shape of the cotyledons in part to the space they occupied in the seeds. GOEBEL (3), however, considered that the form of the seed did not necessarily determine the configuration of the cotyledon. THAYER (9) found in hybrids of *Cucurbita pepo* a high correlation of shape of cotyledons with shape of the seeds from which the cotyledons came, although there was evidence of some normal biparental inheritance also.

Material and methods

Coefficients to express the correlation between shape of cotyledons and shape of fruits were calculated from shape indices. These indices were determined from measurements of cotyledons and fruits. The formula used to find the index numbers of cotyledons was:

$$\text{shape index} = \frac{\text{maximum width}}{\text{maximum length}} \times 1000$$

and that for fruits:

$$\text{shape index} = \frac{\text{greatest equatorial diameter}}{\text{polar diameter}} \times 1000$$

Thus the shape index is 1000 for a spherical fruit, less than 1000 for an elongated fruit, and greater for an oblate fruit. Cotyledons in the types studied always had shape indices less than 1000.

During the summer of 1931 measurements of cotyledons and fruits were obtained for 1169 plants comprising 242 varieties and selections of watermelons and citrons. Besides these, a large number of cotyledons and fruits of 23 selections of Iowa Belle were measured without keeping records of individual plants. The plants were grown in the melon-growing district of Muscatine County, Iowa. The measurements of the cotyledons were made after the first

and second true leaves had formed on most of the seedlings (15 to 22 days after planting), the measurement of one cotyledon of each plant being considered sufficient. Usually only the largest and most perfect fruit from each vine was measured, although in some cases several fruits were used and the results averaged. Measurements were made on the cut surfaces of melons split longitudinally.

Seeds to be measured were selected at random from the packets from which the seeds planted in the field were taken. The index for seed shape was calculated in the same manner as that for shape of cotyledons.

Coefficients of correlation were computed according to the methods outlined by WALLACE and SNEDECOR (10), and the covariance was analyzed by the method given by SNEDECOR (8). For each group of melons three coefficients were computed for the correlation between shape indices of cotyledons and fruits: (1) that for the *total* correlation in individual plants, disregarding varieties; (2) that for the correlation *between* varieties, the correlation in individuals within varieties being eliminated; and (3) that for the correlation *within* varieties after the correlation between varieties had been eliminated. Coefficients were tested for significance by referring to the tables of WALLACE and SNEDECOR.

Investigation
SHAPES OF COTYLEDONS AND FRUITS

There are numerous intergrading shapes in cotyledons of watermelons, the chief variation being in the ratio of maximum width to maximum length. The range in form is from short oval or slightly ovate to long elliptical (figs. 1–8). The indices of the more noticeably oval forms vary from about 690 to 750 or higher, while those of the long types range from 625 to 550 or lower. The mean shape index of the cotyledons of 1077 seedlings of 243 varieties and selections was 655. In the Iowa Belle variety the blade of the oval type of cotyledon is more or less rounded at the base, whereas the elongated type has a tapering or wedge-shaped base (figs. 1–5, 8). In very young Iowa Belle seedlings, oval cotyledons have a rugose or crimped appearance which is lacking in the elongated ones (fig. 8). This distinction disappears as the plants get older.

The variations in shape of watermelon fruits are more extreme than those of the cotyledons. In several varieties the melons are almost spherical or slightly oblate; that is, with shape indices of 1000 or more. On the other hand, certain long-fruited varieties have shape indices below 500.
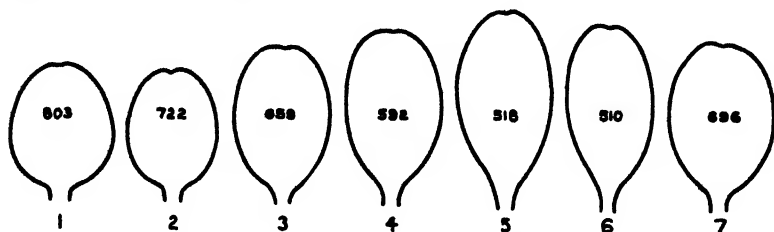
FIGS. 1–7 —Outlines of watermelon cotyledons of different shapes with the shape index of each figs 1–5, Iowa Belle, fig 6, Sugar Stick, fig. 7, White Seeded Chilian.
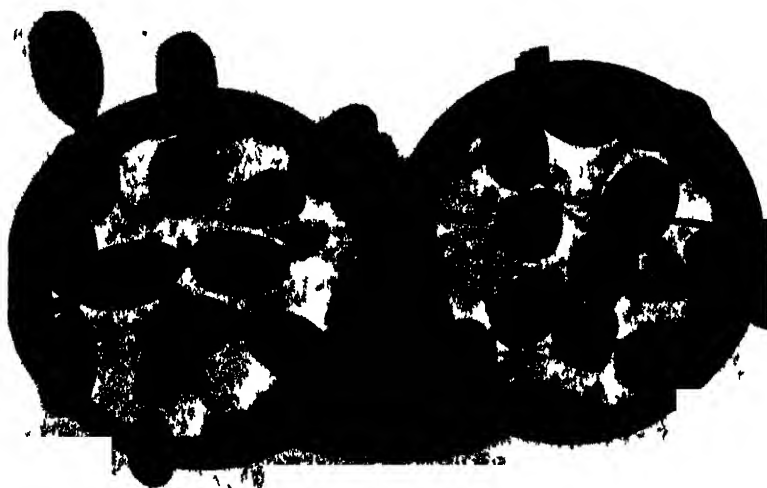
FIG. 8 —Watermelon seedlings showing shape and texture of the cotyledons· left, Kleckley Sweet with long type of cotyledons; right, Round Iowa Belle.

CHANGES IN SHAPE OF COTYLEDONS WITH DEVELOPMENT

In order to ascertain whether or not the cotyledons change in shape as they develop, measurements were taken at two different ages on a number of plants in the greenhouse. The first reading was taken when the plants were at about the stage shown in figure 8,

before true leaves had developed. The second measurements were taken 12 days later at which time the second true leaf was in evidence on most seedlings. A total of 189 plants was used in the first reading and 192 in the second, the increase in number being due to the fact that a few plants were too young to be measured at the first reading. These plants represented 24 different varieties. The mean shape index of the cotyledons was 674 at the time of the first measurements, but this mean had changed to 654 at the time of the second reading, there being a difference of 20. FISHER's (1) method of testing the difference between two means shows this difference to be highly significant, the value of t being 3.78 whereas a t of only 2.59 would have given a probability of 0.01. The samples used were too small to test the significance of the change in each variety, although in every variety but one the mean shape index became smaller. In the one exception the index remained the same. These data indicate that in general the cotyledons of watermelons change in shape as they develop, the increase in length being comparatively greater than the increase in width. This study was not carried far enough to determine at what age the cotyledons assume a constant shape. It is interesting to compare these results with those of HILL (5), who found that in the cotyledons of *Digitalis* the ratio of width to length increased with age instead of decreasing.

CORRELATION OF SHAPE OF COTYLEDONS WITH SHAPE OF FRUITS

CORRELATION IN WATERMELONS.—The data showing correlation of shape of cotyledons with that of fruits in different groups of melons are summarized in table I. As was mentioned earlier in this paper, the methods of analysis of covariance were employed so as to obtain correlation coefficients, not only for the *total* correlation of the individuals of a group but also for the correlation *between* varieties and for that *within* varieties. In all groups of watermelons the coefficients for the total correlation of indices of cotyledon shape and fruit shape were highly significant except in group 6, which contained only 30 plants. The coefficients for the correlation between varieties were much higher than those for total correlation in all groups. The correlation within varieties was much lower than the total but was significant in all watermelons except groups 6 and 7.

Group 1 consisted of 81 inbred selections of the Iowa Belle variety. Some of these selections were homozygous for spherical fruits, some homozygous for elongate fruits, and some were heterozygous for fruit shape. Individual records were kept on 360 plants. These included

TABLE I

CORRELATION BETWEEN SHAPE OF COTYLEDONS AND SHAPE
OF FRUITS IN DIFFERENT GROUPS OF MELONS

| GROUP | DESCRIPTION | NUMBER OF PLANTS | VARIETIES AND SELECTIONS | MEAN SHAPE INDEX | | CORRELATION COEFFICIENTS | | |
				COTYLEDONS	FRUITS	TOTAL	BETWEEN VARIETIES	WITHIN VARIETIES
1...	Inbred Iowa Belle	360	81	648±2	763± 6	0 72†	0 88†	0.21†
2...	Inbred Iowa Belle	.....	23	650±7*	722±30*	.....	0.96†	.....
3..	American varieties	164	28	664±3	763±10	0 58†	0.72†	0.29†
4...	Foreign varieties (includes 10 American varieties)	398	89	663±2	817± 6	0.48†	0 60†	0.16†
5..	American and foreign (groups 3 and 4)	562	117	663±1	801± 5	0 51†	0.63†	0.20†
6...	Other inbreds	30	6	637±6	713±28	0.40‡	0.66	0 07
7...	F₁ hybrids	82	14	652±5	670±13	0.79†	0 95†	0.03
8...	F₂ hybrids	43	5	621±5	602±15	0.70†	0.99†	0.38‡
9...	All watermelons (groups 1, 3, 4, 6, 7, 8)	1077	223	655±1	768± 4	0.61†	0.75†	0.20†
10...	Citrons	59	13	638±3	870±20	0.00	0.04	−0.07
11...	Watermelon-citron hybrids	33	6	651±6	603±17	0.14	0.49	−0.09
12...	Cucumis melo	55	19	513±4	802±15	0.27‡	0.31	0.17

* The mean of the varietal means.
† Highly significant, that is, with probability of 0.01 or less.
‡ Statistically significant, with probability between 0.05 and 0.01.

almost every variation in fruit shape as well as in cotyledon shape. Group 2 consisted of 23 of the best selections of the Iowa Belle which were grown in large numbers for breeding purposes. These were mostly homozygous for round or long fruit shape. Measurements were taken on a number of plants in each selection without keeping records of individuals. From these, the mean shape indices of the cotyledons and of the fruits were calculated for the different selec-

tions. The correlation coefficient calculated from these means was 0.96, which was highly significant as judged by the statistical test.

Group 3 was composed of 28 American varieties of watermelons (listed in table II), and group 4 contained 10 American and 79

TABLE II

MEAN SHAPE INDICES OF COTYLEDONS, SEEDS, AND FRUITS OF 28 AMERICAN COMMERCIAL VARIETIES OF WATERMELONS

VARIETY	COTYLEDONS		SEEDS		FRUITS	
	N	SHAPE INDEX	N	SHAPE INDEX	N	SHAPE INDEX
Angeleno, White Seeded.......	18	697± 5	20	602±6	6	877±16
Arikara....................	24	668± 6	20	593±5	7	827±17
Chilian, Black Seeded........	25	685± 5	20	592±4	5	923±25
Chilian, White Seeded........	26	693± 4	20	607±4	7	924±14
Climbing...................	18	700± 8	20	651±7	5	993± 5
Cole's Early................	20	693± 6	20	621±7	7	829±29
Excel......................	28	580± 7	20	592±5	7	552±18
Field's Jumbo..............	28	680± 4	20	605±6	6	803±14
Florida Favorite............	18	601± 6	20	573±5	5	502±12
Fordhook Early.............	17	734± 6	20	601±6	7	832± 5
Golden Honey..............	17	698± 7	20	614±6	7	812±27
Ice Cream..................	21	653± 5	20	592±5	5	504±19
Kleckley Sweet..............	29	628± 4	20	605±5	5	489±13
Klondike...................	24	685± 6	20	630±4	3	705±33
Mammoth Ironclad..........	21	683± 5	20	586±5	6	600±19
Mountain Sweet.............	23	672± 6	20	603±5	6	878±20
New Wonder................	17	597± 5	20	591±6	4	552± 8
Phinney's Improved..........	14	696± 8	20	597±4	5	632± 9
Princess....................	22	651± 4	20	665±8	7	952±12
Rattlesnake.................	26	584± 6	20	561±6	5	611±29
Stone Mountain.............	20	690±11	20	607±6	6	903±37
Sugar Stick.................	23	556± 6	20	593±6	7	497±13
Sun, Moon, and Stars........	23	662± 6	20	621±4	7	817±46
Sweetheart.................	22	684± 5	20	642±6	6	856±24
Sweet Siberian..............	19	623± 8	20	574±4	5	773±58
Thurmond.Gray.............	20	636± 6	20	575±5	5	536±11
Will's Sugar................	24	643± 5	20	647±8	6	904±18
Winter.....................	19	707± 4	20	662±6	7	968±10

foreign varieties. Group 5 was the combination of groups 3 and 4. The correlation coefficients of these groups were lower than those for Iowa Belle (table I). It should be pointed out that the number of individuals listed under "fruits" in table II applies to both cotyledons and fruits for the correlation of shape of cotyledons with shape of fruits. Measurements from plants grown in the greenhouse were

added to those from the field to obtain the numbers under "cotyledons" in table II.

Group 6 was a miscellaneous lot of inbreds consisting of three selections of Kleckley Sweet, one of a Japanese variety, and two of a French variety. The 82 plants of group 7 represented the F_1 progenies of 14 different varietal crosses. Group 8 contained F_2 plants of hybrids between Iowa King and Iowa Belle and between Pride of Muscatine and Iowa Belle. The coefficients for total correlation and for correlation between selections of these hybrids are very high.

Group 9 was the combination of all the other groups of watermelons except group 2. In this total number of 1077 plants, coefficients for the correlation between indices of cotyledon shape and fruit shape were as follows: total correlation, 0.61; between varieties, 0.75; within varieties, 0.20.

The fact that the correlation between varieties in watermelons was generally high, whereas that within varieties was low, indicates that for each variety there is a characteristic cotyledon shape associated with a characteristic fruit shape, but that within these varieties there are many individual variations in shapes of cotyledons and fruits which are unrelated. The general impression which one gets in observing and working with the plants coincides with these results. The data indicate that it is possible to forecast the mean shape of fruits of a variety or selection by averaging a number of cotyledon indices, but that the probability of making accurate predictions for individual plants is considerably smaller. In practical experience, it has been possible to predict the general shape of fruits of selections of Iowa Belle merely by observing the shapes of the cotyledons without making measurements. Also, the homozygosity of fruit shape in a selection can be foretold by observing the uniformity of cotyledon shape.

CORRELATION IN CITRONS AND WATERMELON-CITRON HYBRIDS.— In all groups of watermelons, indices of the cotyledon shapes were significantly and positively correlated with the indices of fruit shapes. This was not true in the citrons studied. In 59 plants, representing 13 varieties, the correlation coefficients were practically zero (table I, 10). In a group of watermelon-citron hybrids the coefficients were not significant (table I, 11).

CORRELATION IN CUCUMIS MELO.—The coefficient for the total correlation between indices of cotyledon shape and fruit shape in 55 plants of *Cucumis melo* was 0.27, which was statistically significant. The correlation between varieties and that within varieties was not significant (table I, 12). It is interesting to note that the correlation between varieties was larger, and that within smaller, than the total, as was the case in all groups of watermelons. This group of cantaloupes was a very heterogeneous one and included plants from 19 different varieties.

CORRELATION OF SHAPE OF SEEDS WITH SHAPE OF COTYLEDONS AND WITH SHAPE OF FRUITS

The correlation between shape of cotyledons and shape of fruits in watermelons suggested that these characters might also be correlated with the shape of the seeds from which the plants were grown. Shape indices were calculated for 20 seeds from each of the 28 varieties listed in table II. Mean indices of cotyledons were obtained by averaging the indices of a number of plants grown in the greenhouse with those grown in the field. The correlation coefficient was calculated from these mean shape indices of cotyledons and seeds. This coefficient was 0.42 and was statistically significant. The mean shape indices of seeds (table II) were paired with the mean shape indices of fruits to determine the degree of correlation between shape of seeds and shape of fruits. The coefficient (0.60) was highly significant, indicating that varieties of melons with spherical fruits have seeds which are less elongated than those from long-fruited varieties.

Summary

1. The correlation of shape of cotyledons with shape of fruits, of shape of seeds with shape of fruits, and of shape of seeds with shape of cotyledons was investigated in melons.

2. Coefficients of correlation were calculated by using shape indices. By employing the methods of analysis of covariance, coefficients were determined, not only for the total correlation of variations in shape but also for the correlation between varieties and for that within varieties.

3. Shape of cotyledons was significantly and positively correlated

with shape of fruits in watermelons, the coefficients for the total correlation ranging from 0.40 to 0.75 and those for the correlation between varieties from 0.60 to 0.99.

4. There was no significant correlation of shape of cotyledons with shape of fruits in citrons nor in watermelon-citron hybrids.

5. Shapes of cotyledons were found to be significantly correlated with shapes of fruits in a small group of plants of *Cucumis melo* L. ($r = 0.27$).

6. In 28 American varieties of watermelons, the mean shape indices of seeds were significantly correlated with mean shape indices of cotyledons ($r = 0.42$) and with mean shape indices of fruits ($r = 0.60$).

7. A significant change in the shape of watermelon cotyledons occurred as the seedlings grew, the relative length of the cotyledons increasing more rapidly than the width.

8. In many varieties of watermelons, and particularly in the Iowa Belle variety, fruit shape can be foretold from observations on the cotyledons. There is much greater accuracy in forecasting the mean shape of fruits of a variety or selection than in forecasting the fruit shape for an individual plant. Strains homozygous for fruit shape may be selected by observing the uniformity of cotyledon shape.

LITERATURE CITED

1. FISHER, R. A., Statistical methods for research workers. 4th revised ed. Edinburgh. 1933.
2. FOCKE, W. O., Die Pflanzen-mischlinge. Berlin. 1881.
3. GOEBEL, K., Organography of plants. Authorized English ed. Part II, p. 405. Oxford. 1905.
4. GROTH, B. H. A., The F_1 heredity of size, shape, and number in tomato leaves. Part I. Seedlings. New Jersey Agr. Exp. Sta. Bull. 238. 1911.

5. HILL, J. B., Cotyledon form and size in reciprocal hybrids between species of *Digitalis*, BOT. GAZ. 80:84–92. 1925.

6. HUTCHINS, A. E., Morphological relationships in the ontogeny of the cultivated cucumber, *Cucumis sativus* L. Minnesota Agr. Exp. Sta. Tech. Bull. 96. 1934.

7. LUBBOCK, SIR JOHN, A contribution to our knowledge of seedlings. Vol. I. pp. 8, 602–604. London. 1892.

8. SNEDECOR, G. W., Calculation and interpretation of analysis of variance and covariance. Ames, Iowa. 1934.

9. THAYER, G. B., Inheritance of cotyledonary characters in *Cucurbita pepo*. Bull. Torr. Bot. Club 61:263–269. 1934.

10. WALLACE, H. A., and SNEDECOR, G. W., Correlation and machine calculation. Revised. Iowa State Coll. Off. Publ. Vol. 30. no. 4. Ames, Iowa. 1931.

SETCREASIA BREVIFOLIA, A FURTHER EXAMPLE OF POLYPLOIDY AND STRUCTURAL HYBRIDITY IN THE TRADESCANTIAE

M. MARGARET RICHARDSON

(WITH SEVEN FIGURES)

Introduction

The occurrence in the Tradescantiae of polyploidy and of structural hybridity, in some cases associated together, is well known (for example, *Rhoeo discolor, Zebrina pendula, Tradescantia bracteata,* and *T. virginiana*) and has been fully discussed by DARLINGTON (2), KOLLER (8), and others. The observations recorded here show that a form of *Setcreasia brevifolia* Rose exhibits both tetraploidy and structural hybridity, with configurations strikingly similar to those of *T. virginiana* (2, 8).

The large size of the chromosomes, together with the small number and terminal position of the chiasmata in this material, makes this species suitable for a quantitative analysis of chromosome behavior in polyploid associations.

Material and methods

Chromosome observations in meiosis and in the first division of the pollen grain were made from aceto-carmine and smear preparations. The latter were fixed in 2 BE and stained in gentian violet. I am indebted to Mr. S. O. S. DARK for his illustrations of meiosis in the diploid ($\times 4470$) and of metaphase chromosomes in pollen grains ($\times 3300$). Illustrations of meiotic chromosomes in the tetraploid were drawn from aceto-carmine preparations at a magnification of $\times 3300$.

The origin of the tetraploid form is unknown, but it is undoubtedly a member of the genus *Setcreasia;* and although morphologically distinct from the diploid form of *S. brevifolia*, being smaller in habit, it agrees with this species more closely than with any other member and may therefore be considered a variety. *Setcreasia brevifolia* has been variously named *Treleasia* and *Neotreleasia brevifolia.*

Observations

SOMATIC CHROMOSOMES

There are two forms of *Setcreasia brevifolia;* one form has 2n = 12 and the other is a tetraploid with 2n = 24. The chromosomes are large with median or submedian spindle attachments, and show little variation in size. Metaphase chromosomes in the first division of the pollen grain are shown in figures 1 and 2. DARLINGTON (2, p. 214) examined somatic metaphase divisions in the root tip of the tetraploid form, and reports that the chromosomes closely resemble some varieties of *Tradescantia virginiana*, but that there are three or four chromosomes with constrictions more nearly terminal than is usual in that species.

TABLE I

CHIASMA FREQUENCY AND TERMINALIZATION COEFFICIENT IN THE DIPLOID

	NO. OF NUCLEI	NO. OF BIVALENTS	TOTAL NO. OF CHIASMATA	MEAN NO. OF CHIASMATA PER NUCLEAR COMPLEMENT	MEAN NO. OF CHIASMATA PER BIVALENT	TOTAL NO. OF TERMINAL CHIASMATA	TERMINALIZATION COEFFICIENT
Diakinesis.......	5	30	86	17 2	2 86	20	0.23
Metaphase......	4	24	63	15 75	2.62	36	0 57

MEIOSIS IN THE DIPLOID

Meiosis was regular in the diploid form (fig. 3), and configurations very similar to those of *Spironema fragrans*, another member of the Tradescantiae, were found at diakinesis and metaphase (11).

The number of chiasmata varies from 3 to 5 per bivalent at diakinesis, with a mean of 2.86. There is a slight reduction in the number of chiasmata at metaphase, and the proportion of terminal associations is increased (table I). No attempt was made to distinguish between different kinds of bivalents.

MEIOSIS IN THE TETRAPLOID

Meiotic behavior in the tetraploid form is strikingly similar to that in *Tradescantia virginiana*, both in size and form of metaphase configurations and in the average number of quadrivalents per

nuclear complement. It differs from *T. virginiana* in that there is a higher degree of interstitial chiasmata and that fewer types of quadrivalent and trivalent associations requiring quadruple and triple chiasmata are present. Interstitial chiasmata are sometimes found in *T. virginiana*, but only comparatively infrequently, and
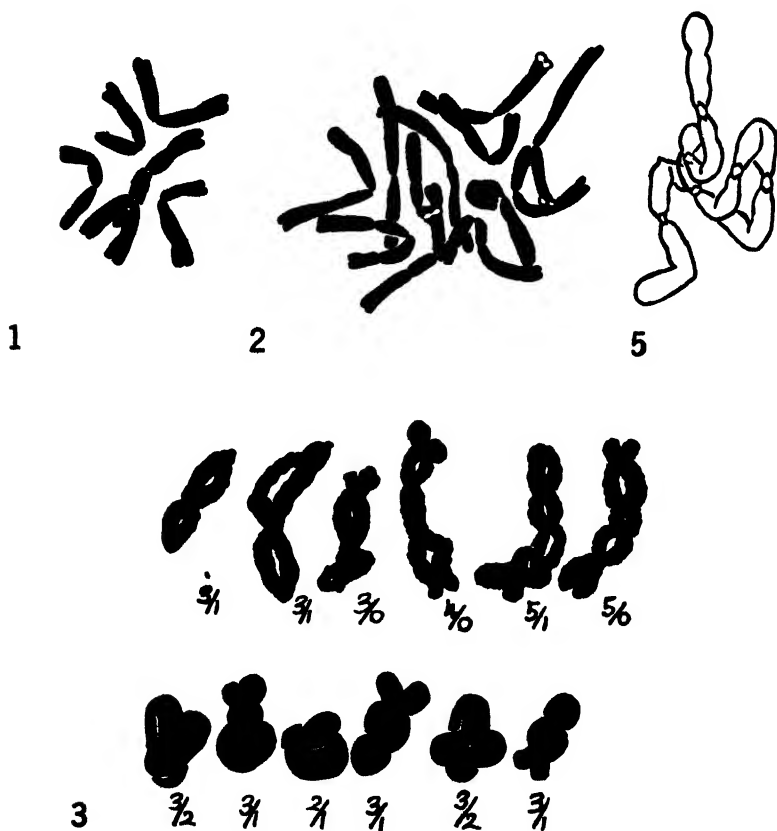
FIGS. 1–3, 5.—Fig. 1, first pollen grain division (n=6); fig 2, same (n=12); fig. 3, meiosis in diploid form (diakinesis above, metaphase below); fig. 5, chain of 8 in the tetraploid form.

have been regarded by DARLINGTON (2) and KOLLER (8) as evidence of non-homology of parts of pairing chromosomes. DARLINGTON reports that three or four quadrivalents with six or four bivalents were most commonly found to comprise the nuclear comple-

ment in *T. virginiana;* all variations from one to six quadrivalents were present, and a few univalents, usually associated with trivalents, were observed. In *Setcreasia brevifolia* all variations of association from five quadrivalents and two bivalents to eleven bivalents and two univalents were seen, there being most frequently two or three quadrivalents. Trivalents are present only in small numbers (table II) and the number of univalents greatly exceeds the number of trivalents. One or two examples of interlocking were found (fig. 4).

TABLE II

FREQUENCY OF DIFFERENT TYPES OF CHROMOSOME
ASSOCIATION IN THE TETRAPLOID

TOTAL NO. OF CELLS	NO. OF CHROMO-SOMES	TYPES OF ASSOCIATION WITH DIFFERENT NUMBERS OF CHIASMATA									
		viii		vi	iv		iii		ii		i
		8	7	6	4	3	3	2	2	1	
72	1728	1	1	1	96	67	1	28	229	190	129
Total		2		1	163		29		419		129

The relative scarcity of multiple chiasma associations is correlated with a low chiasma frequency and may indicate interference (tables III, IV). Only one bivalent with three chiasmata was seen in about 100 nuclei. One example of a quadruple chiasma was found, and six of triple chiasmata; four in quadrivalents and two in trivalents. These all required the formation of three chiasmata in a chromosome (figure 6 illustrates the types of chromosome association observed). Thus only six chromosomes out of a total of 1728 in the 72 cells analyzed showed the presence of three chiasmata, the remainder having two, one, or none (table IV). Quadrivalents, with the preceding exceptions, were found to be either rings or chains (table II) of roughly equal proportions, and trivalents were chains. Ring quadrivalents most commonly segregate disjunctionally, but several cases of two adjacent chromosomes going to the same pole were observed. Figure 5 shows one case of a chain of eight chromosomes.
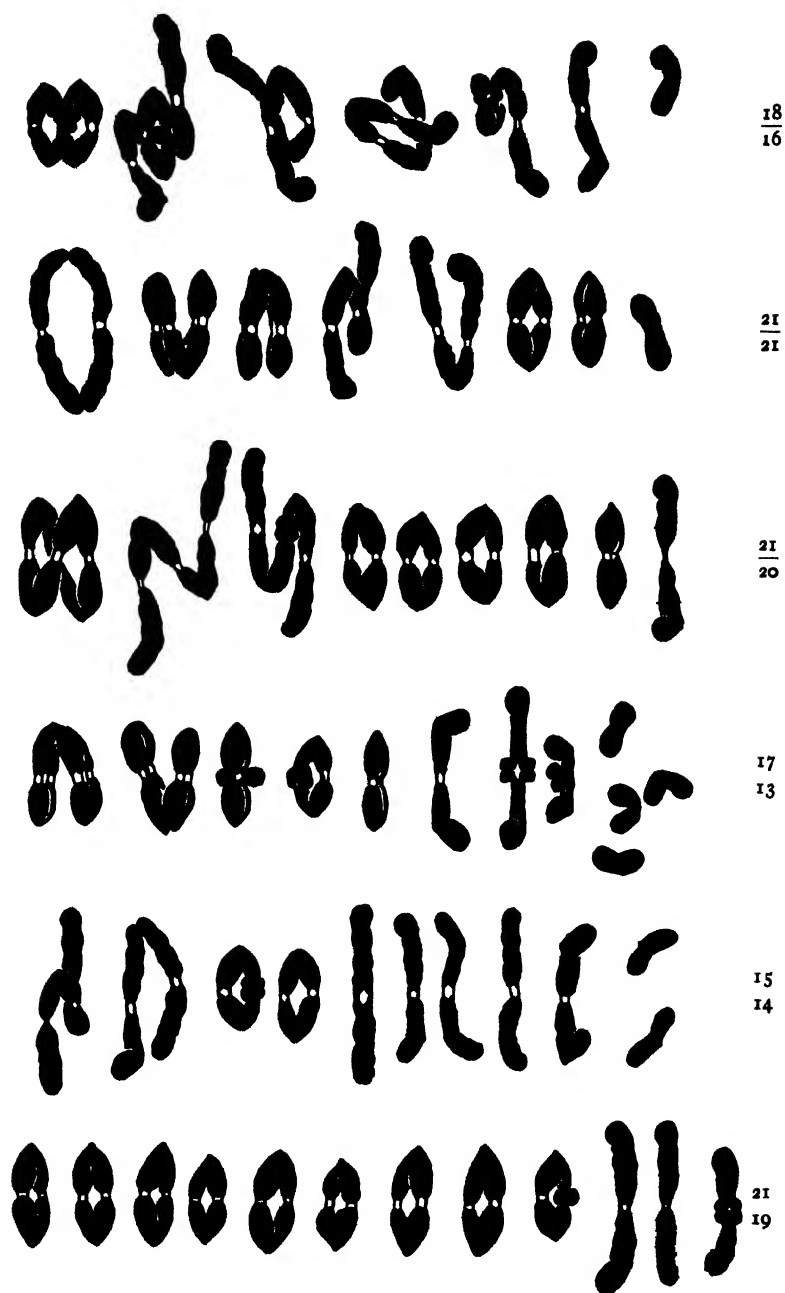
FIG. 4.—Meiosis in the tetraploid form; whole nuclei showing different types of chromosome association in side views of metaphase, and numbers of whole chiasmata.

In following chiasma behavior quantitatively, the method used by DARLINGTON and MATHER (5) for triploid *Tulipa*, whereby "half chiasmata" are counted, has been adopted. Chiasma frequency is thus analyzed for each chromosome and not for different configurations. There are two possible ways of scoring the frequency of half

TABLE III

HALF CHIASMA FREQUENCY FOR WHOLE NUCLEAR COMPLEMENT AND PROPORTION OF TERMINAL ASSOCIATIONS OF WHOLE CHIASMATA (TETRAPLOID)

TOTAL NO OF NUCLEI	TOTAL NO OF CHROMO-SOMES	HALF CHIASMA FREQUENCY FOR WHOLE NUCLEAR COMPLEMENT											TOTAL NO OF HALF CHIAS-MATA	MEAN NO OF HALF CHIAS-MATA PER COM-PLE-MENT	MEAN NO OF HALF CHIAS-MATA PER CHRO-MO-SOME	TOTAL NO WHOLE CHIAS-MATA	TOTAL NO WHOLE TERMI-NAL CHIAS-MATA	TERMI-NALIZA-TION CO-EFFI-CIENT
		26	28	30	32	34	36	38	40	42	44	46						
72	1728		1	3	6	14	20	11	6	10	1		2628	36 3	1 51	1314	1265	0 96

TABLE IV

PERCENTAGE FREQUENCIES OF CHROMOSOMES WITH DIFFERENT NUMBERS OF HALF CHIASMATA (TETRAPLOID)

TOTAL NO OF CHRO-MOSOMES	TOTAL NO OF HALF CHIAS-MATA	HALF CHIASMA FREQUENCY			
		0	1	2	3
1728	2628	7 4	33 3	58 8	0 3

chiasmata, according to the genetical interpretation of a chiasma; these have been adequately discussed by DARLINGTON and MATHER. In the present analysis, half chiasmata have been counted in agreement with the modified chiasmatype theory (3). Thus a chain of four is scored as having 1, 2, 2, 1 half chiasmata per chromosome, and a quadrivalent of the type 2 (fig. 6) 1, 2, 3, 2 half chiasmata per chromosome (fig. 7).

Dissimilar chromosomes are not distinguishable during meiosis,

so that they have all been classed together; but since only small size differences are present, they may be expected to show similar chiasma frequencies. It is found that there is a mean half chiasma frequency of 1.5 per chromosome (table III). The number of chiasmata per chromosome is therefore much reduced from the diploid.

EVIDENCE OF STRUCTURAL HYBRIDITY

The probable presence of structural hybridity in this tetraploid form is shown, not only by associations of more than four inter-

6
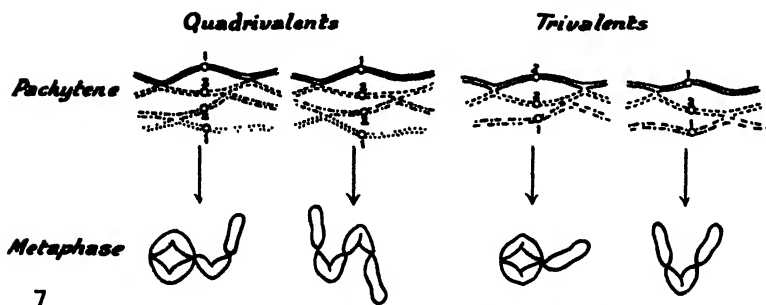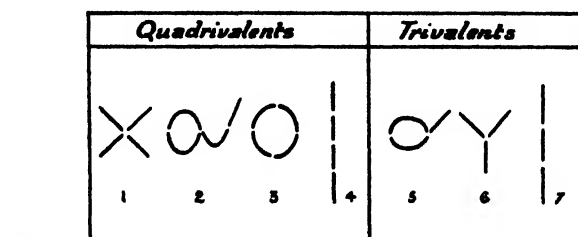
FIGS. 6, 7.—Diagrams illustrating half chiasmata

stitial chiasmata and low chiasma frequency, but also by the presence of trivalents and the large excess of univalents. Although chiasma frequency is unknown at the early stages, it may be deduced to be low from the infrequency of multiple chiasmata.

Trivalents are not usually found in pure autopolyploids, or only very rarely, and potential quadrivalents are replaced by bivalents and univalents (*Primula sinensis*, 3; *Solanum lycopersicum*, 9; *Solanum esculentum*×*racemigerum*, 1; *Avena*, 4). On the other hand

trivalents are present in small proportions in tetraploid interchange heterozygotes (*Tradescantia virginiana*, *Rosa relicta*, 6; *Anthoxanthum odoratum*, 7; *Aucuba japonica*, 10).

If pairing is complete and there is only one exchange of partner between the four chromosomes in an autotetraploid, trivalents may be formed only in two-thirds of the cases when there are two chiasmata per association; or when there are more than two chiasmata, if two lengths of paired chromosomes fail to form chiasmata and one chromosome has all the chiasmata in the association. If one length of paired chromosomes is sufficiently long to form two or more chiasmata, it seems unlikely that the other arm of equal length in this configuration will fail to form any. Similarly, if there are two exchanges of partner between the four chromosomes, trivalents may be formed only in cases when the chiasma frequency per association is low and there are three or four paired lengths of chromosomes without chiasmata.

When four pairing chromosomes are structurally differentiated, the frequency of trivalent formation may be increased for two reasons: (1) Greater mechanical difficulties of pairing, leading to unsynapsed parts of chromosomes and hence reduction in the number of pairing blocks. (2) A greater variation in pairing, for if structural interchange between chromosomes took place previous to chromosome duplication, there will be four pairs of similar chromosomes of the following constitution: 2 AA′, 2 AB′, 2 BA′, and 2 BB′. Or if subsequently, there will be two sets of three similar chromosomes and two dissimilar chromosomes: 3 AA′, 1 AB′, 1 BA′, and 3 BB′. In both cases increased opportunities for trivalent formation are found. There is no evidence to show which has occurred.

The presence of single interstitial chiasmata instead of loops indicates that the interchanges are probably at the ends of the chromosomes. The occurrence of four interstitial chiasmata in a single nucleus, and the ring of eight, show further that there was at least one interchange, if this took place before the number of chromosomes was duplicated; or that there have been more, if structural hybridity has followed tetraploidy.

In view of the differences in cytological behavior, it seems probable that the tetraploid form is specifically distinct from the diploid.

Summary

1. Meiosis is followed in two forms of *Setcreasia brevifolia* Rose. Behavior is normal in the diploid (2n = 12) and bivalents show five to two chiasmata at diakinesis, with a slight reduction in number at metaphase and an increase in the number of terminal associations.

2. Behavior in the tetraploid is very similar to that in *Tradescantia virginiana*, and the same types of associations are found Chiasma frequency is reduced, and potential quadrivalents are replaced by a small proportion of trivalents, bivalents, and univalents.

3. Structural hybridity is indicated by associations of more than four, by interstitial chiasmata, and by trivalents.

4. A quantitative analysis is made of the behavior of the tetraploid from 72 nuclei.

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LITERATURE CITED

1. AFIFY, A., The cytology of the hybrid between *Lycopersicum elculentum* and *L. racemigerum* in relation to its parents. Genetica 15:225–239. 1933.

2. DARLINGTON, C. D., Chromosome behaviour and structural hybridity in the Tradescantiae. Jour. Genetics 21:207–286. 1929.

3. ———, Meiosis in diploid and tetraploid *Primula sinensis*. Jour. Genetics 24:65–96. 1931.

4. ———, The origin and behaviour of chiasmata. IX. Diploid and tetraploid *Avena*. Cytologia 5:128–134. 1933.

5. ———, and MATHER, K., The origin and behaviour of chiasmata. III. Triploid *Tulipa*. Cytologia 4:1–15. 1932.

6. ERLANSON, EILEEN W., A group of tetraploid roses in central Oregon. BOT. GAZ. 91:55–64. 1931.

7. KATTERMANN, G., Über die Bildung polyvalenter Chromosomenverbände bei einigen Gramineen. Planta 12:732–774. 1931.

8. KOLLER, P. C., Further studies in *Tradescantia virginiana* var. *humilis* and *Rhoeo discolor*. Jour. Genetics 26:81–96. 1932.

9. LESLEY, M. M., and LESLEY, J. W., The mode of origin and chromosome behaviour in pollen mother cells of a tetraploid seedling tomato. Jour. Genetics 22:419–425. 1930.

10. MEURMAN, O., *Prunus laurocerasus* L., a species showing high polyploidy. Jour. Genetics 21:85–94. 1929.

11. RICHARDSON, M. MARGARET, The origin and behaviour of chiasmata. X. *Spironema fragrans*. Cytologia 5:337–354. 1934.

MORPHOLOGY OF AN INTERNAL TYPE OF ABNORMALITY IN THE FRUIT OF THE PEPPER[1]

H. L. COCHRAN

(WITH FOURTEEN FIGURES)

Introduction

The fact that an internal type of abnormality (fig. 1) occurs rather frequently in the fruit of the pepper, *Capsicum frutescens* L., was pointed out by the writer (2) in a previous publication. Since this
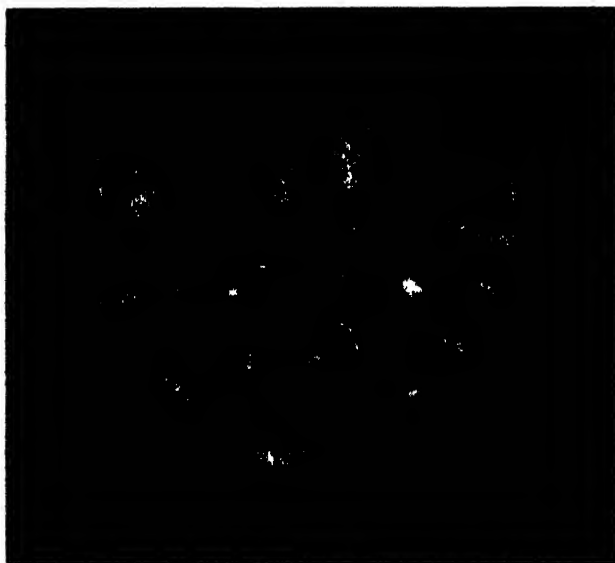
FIG. 1.—Fifteen separate and distinct internal abnormalities from a single pepper fruit.

time a morphological study of the abnormality has been undertaken, primarily to determine, if possible, something of its origin and ontogeny. Facts bearing on this problem seem of economic importance from the point of plant improvement, for such affected fruits usually develop parthenocarpically. Apparently no similar morphological

study of the pepper fruit has been reported, although descriptions of the gross morphology of this peculiar malformation do appear in the literature (18, 1, 10, 17, 7, 13, 6, 11, 19, 15, 12, 8, 2).

Material and methods

The material for this study was selected from pepper plants grown under controlled temperature conditions (60°–70° and 70°–80° F.) in the greenhouses of the Department of Vegetable Crops during the winter of 1932–33. Collections were made at intervals ranging from the early bud stage until fruit maturity, in order that any existing internal abnormalities might be found in their various stages of development. Karpechenko's chrom-acetic killing and fixing solution was used with good success in all cases. The usual laboratory method of dehydrating and imbedding the material in paraffin was followed. Slides were made of sections cut 8–12 μ thick and stained with Heidenhain's iron-alum haematoxylin. All drawings were made with the aid of a Spencer type camera lucida.

Investigation

GROSS MORPHOLOGY OF NORMAL PEPPER FRUIT

The young bud emerges as a mere protuberance from meristematic tissue in the axils of branches of the plant. As soon as the bud begins to open an aperture is formed at its tip, where the corolla lobes meet. The aperture gradually increases in size and extends downward along the margins of the lobes. Soon after this the lobes spread out, thereby exposing for the first time the immature ovary. This becomes larger and is characteristically shaped according to the variety. The fruit matures in approximately 60 days from anthesis, at which time it possesses a distinct depression at the base and three external furrows, one between each locule, that extend down the sides of the fruit to the apical end. From 300 to 550 seeds, depending on the size of the fruit, are borne on the placental tissue, the number usually being rather equally distributed in each of the three locules.

ORIGIN OF ABNORMALITY

The internal abnormality is hypodermal in origin, being initiated in the outermost layers of cells of the placenta when the pepper bud

ranges from 1 to 3 mm. in diameter. It develops simultaneously with
the ovules and for a while approximates them in most respects. Im-
mediately preceding the development of the abnormality and ovules,
the epidermis of the placenta preserves a uniform surface (fig. 2).
The cells of the epidermis and a layer of hypodermal cells soon dif-
ferentiate from the rest of the placental tissue. The cytoplasm of
these cells is very dense, and each contains a conspicuous nucleus
which nearly fills the entire cell. These cells stain more deeply than
do the adjoining ones of the placenta. It is evident that the abnor-
mality initial differentiates from the subepidermal tissue, and at this
stage cannot be detected from the ovule initials that differentiate in
groups from the same tissue. The abnormal tissue, as well as the
ovules, divides at first only in one direction. The epidermal cells
divide anticlinally. The cells lying between the abnormal initials and
those of the ovules divide less rapidly and soon become compressed
and distorted. At this stage the placenta loses its evenness and be-
comes wavy in outline. The identity of the many ovules and that
of the abnormality as well is manifested as blunt protuberances on
the placenta (fig. 3). Subsequent development of the abnormality
and ovule initials is very dissimilar. The ovulary tissue now divides
in all directions and soon constitutes the beginning of the nascent
ovules, while the malformed tissue develops into a protuberance
which constitutes the ensuing abnormality. The accompanying
ovules abort almost invariably.

ONTOGENY

The young developing abnormality is distinguished early in its life
history by the fact that it grows at an unusually rapid rate as a mass
of lightly stained undifferentiated cells. The epidermis, however,
which consists of a single layer of small, rather isodiametric cells,
is differentiated soon thereafter (figs. 4, 5). The remainder of the
abnormality is made up of a mass of thin walled parenchyma tis-
sue (2). As the abnormality increases in size and cell numbers, its
rate of development decreases; yet it remains in excess of any ac-
companying ovules.

Cell division does not continue at a very constant rate throughout
the abnormal tissue. Because of this various forms are developed
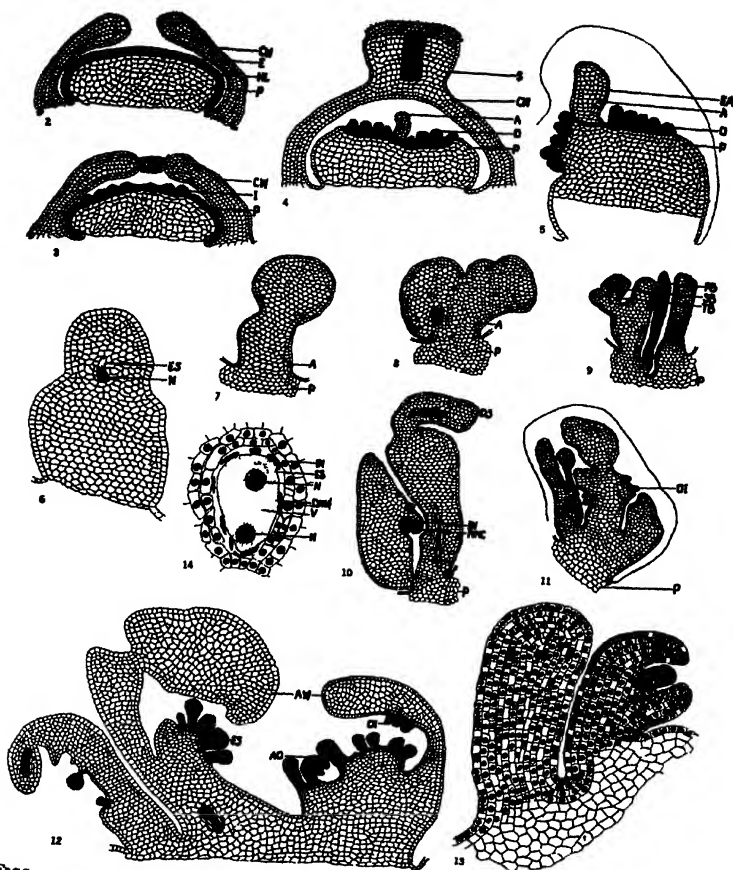
Figs. 2–14.—Fig. 2, longisection through pepper bud 1.5 mm. in diameter showing uniform placental surface prior to ovule and abnormality initiation. ×178. *cw*, carpel wall; *e*, epidermis; *hl*, hypodermal layer; *p*, placenta. Fig. 3, longisection of pepper bud 3 mm. in diameter showing ovule and abnormality initials. ×178. *i*, initials of ovules and abnormality. Fig. 4, longisection through young ovary showing immature ovules and developing abnormality. ×178. *s*, style; *a*, abnormality; *o*, ovules. Fig. 5, longisection of young ovary showing developing abnormality with epidermis differentiated. ×178. *ea*, epidermis of abnormality. Fig. 6, longisection of developing abnormality resembling somewhat a normal ovule in shape and containing an abnormal uninucleated embryo sac. ×750. *es*, embryo sac; *n*, nucleus. Figs. 7, 8, longisections through two abnormalities showing close resemblances of abnormal and placental tissue. ×100. Fig. 9, longisection of abnormality showing primary, secondary, and tertiary branches. ×100. *pb*, primary branch; *sb*, secondary branch; *tb*, tertiary branch. Fig. 10, longisection through developing abnormality showing distorted style and abnormal ovule in megaspore mother cell stage. ×178. *ds*, distorted style; *in*, integument; *mmc*, megaspore mother cell. Fig. 11, longisection of abnormality showing ovule initiation therefrom. ×178. *oi*, ovule initials. Fig. 12, longisection through rather mature abnormality showing ovule initiation from several points, an anatropous ovule, and a 2-celled embryo sac. ×415. *aw*, abnormality wall; *oi*, ovule initials; *ao*, anatropous ovule; *es*, 2-nucleate embryo sac. Fig. 13, enlarged view of anatropous ovule (taken from fig. 12). ×960. Fig. 14, enlarged view (from fig. 12) of 2-nucleate embryo sac from within the abnormality, showing integument, disintegration of nucellus, nuclei, and vacuole. ×960. *dnu*, disintegrating nucellus; *v*, vacuole.

and no definite shape can be assigned them. As far as could be detected, the malformation is always initiated as a single protuberance which later may or may not branch (figs. 7-12). It is not uncommon to find abnormal tissue that branches into two or three portions, some of which subsequently develop similar secondary and tertiary branches. Various other grotesque shapes may appear. One abnormality was found which somewhat resembled a normal ovule containing an abnormal one-celled embryo sac (fig. 6). Some abnormalities produce no style-like structures, while others produce two or even three of them.

A microscopic study of prepared sections and also of living specimens with the wide-field binocular microscope indicates that the abnormality is anatomically the same in most respects as the placenta from which it is derived. Both consist of similar parenchyma tissue that has the same stain reaction and both are capable of initiating and developing ovules. The ovules of the abnormality, however, are not restricted to any particular portion of the tissue but appear to arise at any point thereon (figs. 11, 12). Some of these ovules are apparently normal, as far as size, shape, structure, and certain stages of development are concerned. While it is true that many of the ovules from within the abnormality abort when young, those that develop further are anatropous (figs. 12, 13). This work has revealed but one ovule in what appears to be the megaspore mother cell stage (fig. 10), and none developed further than the 2-nucleate embryo sac stage (fig. 14). Continued development probably occurs rather rarely; at least the ovule rarely reaches maturity. Apparently STURTEVANT (17) is the only person who has reported such an internal type of abnormality which contained seed.

Normal and abnormal 1- and 2-nucleate embryo sacs have been compared and found to be very similar. Even the nucellus of both start degenerating upon reaching approximately the same stage of development. The only exception noted was a difference in time of nuclear division. When using ovule size as a criterion of age, the one from within the abnormality was found to be rather slower in dividing, although the two subsequently became the same.

Discussion

For the past three years the type of internal abnormality reported here has been prevalent to some extent in fruits from plants grown in the greenhouse under controlled temperature conditions. A study has been made of these changes but still the question arises, What explanation can be offered as to the cause of this phenomenon? No observations made in the present study supply a definite answer to this question.

As the affected pepper fruits for the most part develop partheno-carpically, and are therefore partially sterile, the same factor or inter-relation of factors may be responsible for both the seedless condition and the development of the abnormality as well.

DARWIN (3) has pointed out that influences of both environment and hybridity may, through compensations of growth, result in such a condition as seedless fruits. The characters parthenocarpy and embryo abortion in seedless fruits are known to be hereditary, as exemplified in the cucumber by WELLINGTON and HAWTHORN (20) and HAWTHORN and WELLINGTON (9). According to PEYRITSCH (14), however, these conditions may be induced artificially.

Since the plants used in this study were grown in the greenhouse under almost ideal conditions as far as temperature, soil nutrients, and soil moisture are concerned, some vegetative vigor resulted. There seems to be no direct evidence that the seedless type of fruits containing the abnormality arises as a direct response to excessive vegetative vigor, nevertheless ERNST (5) has considered that such characters as apogamy and seedlessness may be initiated under such conditions. STOUT (16) is of the opinion that frequently certain of the unfavorable environmental factors to which a plant is subjected may become sufficiently regular in action to institute a type of sterility. Thus in respect to nutrition there may be a response to such influences as an excess of nitrogen or of water. In general this is characterized by excessive vegetative vigor.

While the preceding evidence on sterility affords no direct explana-tion for the occurrence of the internal type of abnormality, it is believed that the evidence is of enough significance to suggest that hybridity and vegetative vigor as affected by environmental factors may be involved.

Summary

1. The internal type of abnormality occurs frequently in the fruit of the pepper.

2. The abnormality is hypodermal in origin and develops for a while simultaneously with the accompanying ovules. It is initiated when the pepper bud ranges from 1 to 3 mm. in diameter.

3. The young abnormality is distinguished early in its ontogeny by a group of undifferentiated, lightly staining cells which divide very rapidly. The rate of growth, however, decreases with increase in size and age.

4. The epidermis, which consists of a single layer of rather iso-diametric cells, differentiates soon after the preceding stage.

5. No definite shape can be assigned the abnormality since it is so variable. Some contain styles while others do not.

6. The abnormality and the placenta from which it is derived are anatomically the same; both consist of similar parenchyma tissue that takes the same stain, and both are capable of initiating and developing ovules.

7. Most ovules borne in the abnormality abort but those that survive are typically anatropous.

8. One ovule from within the abnormality was found in the megaspore mother cell stage and none were found any further advanced than the 2-celled embryo sac stage.

9. Nuclear division of the embryo sac takes place somewhat slower than that of a normal embryo but subsequently they become identical.

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LITERATURE CITED

1. BORBÁS, V., Fias paprikagy ü möles. Földmüvèlesi èrdekeink **45**:459. 1880.
2. COCHRAN, H. L., Abnormalities in the flower and fruit of *Capsicum frutescens*. Jour. Agr. Res. **48**:737–749. 1934.

3. DARWIN, CHARLES, The variation of animals and plants under domestication. Orange Judd Co., New York 2:205–210. 1868.

4. EAMES, A. J., and MACDANIELS, L. H., Introduction to plant anatomy. McGraw-Hill Book Co., New York. 1925.

5. ERNST, ALFRED, Bastardierung als Ursache der Apogamie im Pflanzenreich. Jena. 1918.

6. GALLARDO, A., Observaciones morfológicas y estadísticas sobre algunas anomalías de *Digitalis purpurea* L. Ann. Mus. Nac. Hist. Nat. Buenos Aires 7:37–72. 1902.

7. HALSTEAD, B. D., A strange thing in peppers. Bull. Torr. Bot. Club 18: 151. 1891.

8. HARRIS, J. A., Prolification of the fruit in *Capsicum* and *Passiflora*. Mo. Bot. Gard. Ann. Rept. 17:133–145. 1906.

9. HAWTHORN, L. R., and WELLINGTON, RICHARD, Geneva, a greenhouse cucumber that develops fruit without pollination. N.Y. State (Geneva) Agri. Exp. Sta. Bull. 580. 1930.

10. HECKEL, E., Nouvelles monstruosités végétales. Bull. Soc. Bot. France (2) 29:292–312. 1882.

11. IRISH, H. C., Variations in some introduced garden vegetables. Soc. Prom. Agr. Sci. Proc. 23:63–64. 1902.

12. MOTTAREALE, G., Gelate e fenomeni cleistogamici e teratologici nel *Solanum melongena* e nel *Capsicum annum* e *C. grossum*. Ann. R. Scula Agr. Portici (2) 6:1–22. 1904.

13. PENZIG, O., Pflanzen-Teratologie, systematisch geordnet. 2 V. (1894 ed.); Aufl. 2, stark verm. 3 V. 1921–22. Berlin. 1894–1922.

14. PEYRITSCH, J., Über künstliche Erzeugung von gefüllten Blüten und anderen Bildungsabweichungen. Sitzb. Wien Akad. 97:597–605. 1888.

15. RAYMONDAUD, E., Endocarpie (inclusion D'un piment dans un piment). Rev. Sci. Limousin 12:369–372. 1904.

16. STOUT, A. B., Types of sterility in plants and their significance in horticulture. Memoirs Hort. Soc. New York 3:3–8. 1927.

17. STURTEVANT, L. E., Seedless fruits. Mem. Torr. Bot. Club 1:14–185. 1890.

18. TERRACCIANO, N., Intorno alla transformazione degli stami in carpelli nel *Capsicum grossum*, e di un caso prolificazione fruttipara nel *Capsicum annuum*. Nuovo Gior. Ital. 10:28–33. 1878.

19. VIVIAND-MOREL, J. V., Gros piment carré, doux, prolifère. Lyon Hort. 24: 382–385. 1902.

20. WELLINGTON, RICHARD, and HAWTHORN, L. R., A parthenocarpic hybrid derived from a cross between an English forcing cucumber and the Arlington White Spine. Proc. Amer. Soc. Hort. Sci. 25:97–100. 1928.

MICROINCINERATION STUDIES: II. LOCALIZATION OF ASH-YIELDING SUBSTANCES DURING MEIOSIS AND ITS POSSIBLE SIGNIFICANCE IN X-IRRADIATION PHENOMENA[1]

FRED M. UBER AND T. H. GOODSPEED

(WITH FOUR FIGURES)

Using the improved microincineration technique of POLICARD (6), SCOTT (7) demonstrated the disposition of inorganic residues in the male germ cells of the white rat and found the major portion of the ash localized in the chromosomes. Later KRUSZYNSKI (5) and others made further observations on several types of cells and on smaller cytological entities with varying success. Previous to these animal studies, FUNAOKA and OGATA (1) had obtained similar results with pollen mother cells of *Vicia faba* in the early stages of division, but their bright-field photomicrographs, taken at a low magnification, combined with their very brief descriptions, give an incomplete and unsatisfying picture. From several standpoints a more detailed investigation of the topography of the dividing plant cell in terms of the disposition, amount, and character of the inorganic salts appeared highly desirable. The bearing of such evidence on the interpretation of the lethal effects of x-rays, its significance for various cytogenetic phenomena, and its intimate relation with the physicochemical organization of the cell immediately come to mind.

Anthers of *Lilium longiflorum* var. *grandiflorum* and of *Kniphofia* sp. were fixed by the Altmann freezing-drying technique, imbedded in paraffin and cut at 7 μ. The merits of this method of fixation as a pretreatment for microincineration as well as a full discussion of the technique itself were presented in previous publications (2, 3, 4), to which the reader is referred also for typical photomicrographs of control material. Sections to be incinerated were placed on microscope

slides without the aid of an adhesive, whereas controls were stained with Heidenhain's iron-alum haematoxylin in the traditional manner. The microincineration apparatus and technique were described at length in the initial paper of this series (11). Usually the time allowed to heat the sections from room temperature to 550° or 600° C. was two hours, the final temperature being maintained for a further period of 2–3 hours. The slides were removed from the oven while hot and the edges sealed at once in order to exclude moisture from the hygroscopic ash. The bicentric dark-field condenser of Leitz with direct current arc illumination was utilized for both visual study and photomicrography, the latter by means of the Leitz "Makam" camera.

The topographical disposition of the inorganic residue following incineration in PMC of *Lilium* is shown in figures 1–4. Striking characteristics of the ash picture are (a) absence of ash in the karyolymph, (b) abundance of mineral residue in the chromosomes, (c) presence of ash in the cytoplasm, and (d) presence of ash in the cell wall. The ostensibly heavy ash deposits at the periphery of the cells are not wholly the product of the cell walls but are due in large measure either to the slime of the anther sac or to a partial collapse of the cytoplasm incident to its dehydration during the freezing process of fixation. Simultaneously certain intercellular spaces were thus created, as evidenced in figures 1–4 (*cf.* also 3, fig. 1). In figures 1 and 2 the dense white ash of the late prophase chromosomes contrasts clearly with the black background representing the karyolymph. Although little is known concerning the chemical composition of the karyolymph, microincineration evidence suggests that it is radically different from that of the cytoplasm. Figures 3 and 4 represent the inorganic residues of the heterotypic metaphase and anaphase respectively. Save for the large quantity of ash in the chromosomes, the spindle regions are devoid of non-volatile mineral substances. A logical interpretation of this evidence would be that the spindle area is formed from the karyolymph of the preceding prophase, a conclusion many cytologists (8) have reached from independent considerations. Residues of telophasic nuclei showed no structure, in general, but only a continuous heavy deposit. The same was true of resting nuclei in the somatic cells of the anthers.

Incinerated sections of anthers of *Kniphofia* sp. gave results comparable with those obtained with *Lilium*, and in addition illustrated most graphically the rich ash content of the tapetum. The tapetum is generally recognized as a nutritive layer (9, pp. 250, 251), so that a high mineral content might well be anticipated. Whether the chromosomal and other ash consists mostly of salts of Ca, Mg, K,
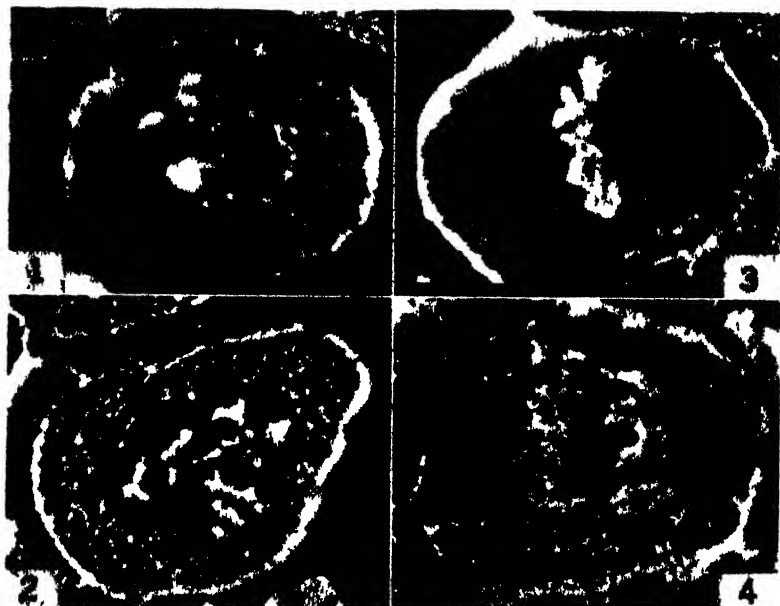
FIGS. 1–4.—Dark-field photomicrographs showing ash distribution in pollen mother cells of *Lilium longiflorum*. Leitz bicentric condenser, N.A. 1.2. 810×. Sections, 7 μ, fixed by Altmann freezing-drying technique: Figs. 1, 2, diakinesis; nuclear cavity clearly delineated, revealing absence of ash in karyolymph; ash conspicuous in bivalents and present in cytoplasm. Fig. 3, heterotypic metaphase plate with ash in chromatin predominant over cytoplasmic residues. Fig. 4, heterotypic anaphase; details of chromosomes well preserved. Note outline of spindle region.

etc., as suggested by several investigators, or is due in large measure to phosphorus, has not as yet been determined. Thus far only gross chemical tests have been made, but these attest the abundant presence of the latter and indicate that a large part of the residue in both cytoplasm and chromatin may result from organic phosphorus compounds. No evidence is furnished on this point by

KRUSZYNSKI (5), who, in making a qualitative chemical analysis of incinerated nerve cells by means of the micromanipulator, apparently did not test for elements other than K, Ca, Fe, and Mg. It is to be noted that nucleic acid, which is known to be present in chromatin and is probably present also in the cytoplasm as a nucleoprotein (cf. 9, pp. 58, 59), has a phosphorus composition of nearly 10 per cent.

The bearing of the fact that elements of comparatively high atomic number are concentrated in the chromosomes on the interpretation of quantitative lethal effects of x-radiation must now be considered. The customary measurement of x-ray dosage in roentgens is based upon the assumption that protoplasm simulates air as regards its mass absorption coefficients. But from the known composition of nucleic acid with its high phosphorus content and the demonstrated presence of non-volatile ash in the chromosomes, a mass absorption coefficient for chromatin at least double that of air would seem a conservative estimate. Although such increased absorption would probably be insufficient to account completely for the known greater sensitivity of nuclei as compared with cytoplasm, it might well be an important contributory factor. The microincineration evidence in itself does not constitute proof on this point, since nuclear injury may conceivably result in a number of ways. According to the quantum theory of biological effects from high frequency radiation, injury occurs when a specific number of quanta are absorbed in a "sensitive volume" of an irradiated object (for relevant literature, see UBER and GODDARD 10). Should chromatin constitute such a "sensitive volume," then it follows that its size would be but a fraction of that calculated for it on the ordinary assumptions.

If the chromosomes possess a relatively great absorptive capacity for high frequency radiation owing to presence of elements of comparatively high atomic weight, then there should be an abrupt quantitative variation in the biological response to x-ray wave lengths on either side of the absorption discontinuities of such elements. Although both quantitative and qualitative wave-length effects have been sought many times in recent years, no investigator, to the writers' knowledge, has explored the "K" discontinuity region

of the elements calcium, potassium, and phosphorus. In the wavelength range concerned, 3–6 Angström units, the obtaining of critical data on biological material with monochromatic x-rays constitutes a difficult task, but one which the writers have planned to undertake in the immediate future.

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LITERATURE CITED

1. FUNAOKA, S., and OGATA, H., Über die Lokalisation der Mineralstoffe in den Zellen. Fol. Anat. Japonica 8:169–171. 1930.
2. GOODSPEED, T. H., and UBER, F. M., Application of the Altmann freezing-drying technique to plant cytology. I. Proc. Nat. Acad. Sci. U.S.A. 20: 495–501. 1934.
3. ——, II. Character of the fixation. Univ. California Publ. Bot. 18:23–32. 1935.
4. ——, and AVERY, P., III. Chromosome structure in *Lilium longiflorum*. *Ibid.* 18:33–44. 1935.
5. KRUSZYNSKI, J., Cytochemische Untersuchungen der veraschten Nervenzelle. Bull. Internat. Acad. Polonaise, Cl. Sci. Math. et Nat. 2:105–116. 1934.
6. POLICARD, A., La microincinération des cellules et des tissus. Protoplasma 7:464–481. 1929.
7. SCOTT, G. H., The disposition of the fixed mineral salts during mitosis. Bull. Histol. Appliq. Physiol. 7:251–256. 1930.
8. SHARP, L. W., Introduction to cytology. p. 136. McGraw-Hill, New York. 1934.
9. TISCHLER, G., K. LINSBAUER's Handb. Pflanzenanat. Bd. II, Teil I. Der Ruhekern. Borntraeger, Berlin. 1934.
10. UBER, F. M., and GODDARD, D. R., Influence of death criteria on the x-ray survival curves of the fungus, *Neurospora*. Jour. Gen. Physiol. 17:577–590. 1934.
11. UBER, F. M., and GOODSPEED, T. H., Microincineration studies. I. Localization of inorganic elements in plant cell walls. Proc. Nat. Acad. Sci. U.S.A. 21:428–433. 1935.

CURRENT LITERATURE

The Algae and Their Life Relations. Fundamentals of Phycology. By JOSEPHINE
E. TILDEN. Minneapolis: University of Minnesota Press, 1935. Pp. xii+550.
Illus. $5.

Instructors presenting a course on the algae have never had a comprehensive
text-book in English to which they could refer the student for material to sup-
plement the lectures and the laboratory work. Professor TILDEN's book is de-
signed to meet this need.

The first chapter is a presentation of the author's hypothesis that the five
classes of algae (Cyanophyceae, Rhodophyceae, Phaeophyceae, Chrysophyceae,
and Chlorophyceae) arose in successive eras, and that the characteristic pigmen-
tation of each class was determined by the intensity of the earth's illumination
during the era in which it appeared. The second chapter (Distribution of algae
in time and space) divides time into the Cyanophycean, Rhodophycean, and
other periods, and holds that variation in illumination is the factor of primary
importance in the present-day distribution of marine algae in the Pacific Ocean.
The third chapter stresses the characteristic pigmentation and food reserves
among the various classes of algae. This chapter is the only place where distinc-
tions are made between the classes of algae. It could have been improved by
pointing out that a lack of sexual reproduction, or fertilization by non-motile
male gametes, or a distinctive type of flagellation in planogonidia and gametes
are of equal importance in distinguishing particular classes.

In the detailed consideration of the various classes, 51 pages are devoted to
the Cyanophyceae, 105 to the Rhodophyceae, 129 to the Phaeophyceae, 15 to
the Chrysophyceae, and 119 to the Chlorophyceae. The chapter on the Rhodo-
phyceae exemplifies the strong and the weak features of the book. The descrip-
tions of the morphology of the various genera take into consideration the most
recent literature and present this accurately. Descriptions of the methods of
harvesting and manufacture of such commercial products as "Irish moss" and
agar add immeasurably to the chapter. They make the student realize that
there is something more to the red algae than carpogonidia and trichogynes, or
haplobionts and diplobionts. On the other hand, starting a discussion of repro-
duction in the Rhodophyceae with the Ceramiales makes a difficult subject even
more difficult. The author's reason for this lies in her belief that the simplest
plants in any group are the most recently evolved ones. It is difficult to find a
justification for this novel hypothesis, either from the known geological history
of other plants, or from that of animals. In connection with the account of each
order there are technical descriptions of the various families and of several

genera in each family. There is no evident reason for the inclusion of these technical descriptions. The book is not designed to serve, and cannot serve, as a manual for the identification of algae. If the generic descriptions are intended to show the student the wide range in structure among the various orders, the same end could have been accomplished in a more effective manner by means of a general discussion. The details of reproduction are described for one or more genera in each family. Sometimes these are given *in extenso* and with numerous illustrations. At other times they are brief and illustrated by means of a life cycle diagram.

The chapters on the brown and the green algae are organized in the same manner and proceed from the complex to the simpler forms. The diatoms are given the same amount of space as the Chinese ideograph meaning algae (a full page text figure and a half page of text).

The author meets the student's confusion arising from the conflicting terminology in phycological literature by proposing a new terminology that is consistent throughout. The most striking innovations (pp. 236–237) are the restriction of the term spore to those produced as a result of meiosis, and the designation of all asexual reproductive bodies as gonidia. Examples of the latter are planogonidia (zoospores), aplanogonidia (aplanospores), and carpogonidia (carpospores). Admitting its greater consistency, such a wholesale introduction of new terms can only further obscure the condition that it aims to clarify. It is to be hoped that the student will consult the papers listed in the extensive bibliography. If he does, he is certain to meet a different terminology and one that must be translated into the terminology of Professor TILDEN's book. Sometimes this will be a difficult matter. For example, one reading the literature on the Phaeophyceae is almost certain to run across the terms "unilocular sporangium" and "plurilocular sporangium." These are not mentioned in the index of the book under review and, as far as the reviewer can find, only once in the text (p. 237). A simpler solution of the whole difficulty would have been the inclusion of a glossary of conflicting terms.

Taken as a whole, the book is an accurate presentation of the present-day factual knowledge of the algae arranged in an unorthodox manner. One cannot deny an author the right to express an opinion contrary to that of a majority of phycologists. However, in a book designed for students it would have been advisable to indicate interpretations that are not in accordance with the opinion of other phycologists. A minor example of this is the treatment of *Botrydium*. The author includes this alga in the Chlorophyceae without hinting that all other phycologists place it in the Heterokonteae.—GILBERT M. SMITH.

Translocation of Solutes in Plants. By OTIS F. CURTIS. New York: McGraw-Hill Co., 1935. Pp. xiii+273.

This new book is probably one of the most important recent contributions to plant physiology. No one is better equipped to treat of the subject than is CUR-

TIS, who some 18 years ago began his researches on solute translocation and who has since encouraged so many other investigators to focus attention on this problem. The book is divided into seven chapters, each dealing with a specific part of the problem: the importance of translocation, the upward transport of organic matter in the phloem, the upward transport of nitrogen and salts through the phloem, the downward transport through the xylem, the criteria and methods of study, the mechanism of movement, and the possible relations between solute movement and plant growth.

The book is written in clear and stimulating style. CURTIS presents all arguments in each chapter, and even though one familiar with the work on translocation will disagree with many of the viewpoints presented, he will subscribe to the author's fairness in his treatment. Perhaps the weakest chapter is that dealing with the upward movement of salts and nitrogen in the phloem. Here, although he presents evidence opposing his views, CURTIS is definitely biased in favor of phloem movement, even though in a later chapter he concedes the possibility of xylem movement. Each chapter is filled with descriptions of experiments dealing with living plants, a pleasant change from the tendency to use mechanical set-ups.

By far the most important and certainly the most brilliant chapter is that dealing with the method of movement. In this chapter of some hundred pages, CURTIS reviews the various hypotheses which have been suggested. His criticisms of these are specific. The hypotheses of MÜNCH, CRAFTS, etc., are first interpreted in the favorable light of their respective authors, then criticised in a most complete and scientific manner. It was rather difficult for the reviewer, however, to leave this very critical method to read about the "protoplasmic streaming hypothesis" which CURTIS seems to favor. While he admits the many attendant difficulties, even pointing out on page 185 that protoplasmic streaming has never been demonstrated in functioning sieve tubes, still in his summarizing statement on page 234 he writes, "The protoplasmic streaming hypothesis seems at present best adapted to meet the requirements of transport and to demand the structure and conditions that obtain in phloem tissue." One is forced to wonder just how scientific is intuition!

The book includes thirteen figures and twenty-four tables, as well as twelve pages of selected literature citations. Each chapter is tersely summarized.— HARRY F. CLEMENTS.

Plant Physiology. By MEIRION THOMAS. Philadelphia: P. Blakiston's Sons and Co., 1935. Pp. vii+494. figs. 57.

This volume is a worthy addition to the list of summaries of this field which have appeared recently. The book, intended for students who already have a general knowledge of the subject, is arranged in a logical order. Part I is concerned with protoplasm, and also contains a discussion of enzymes. Absorption, translocation, and elimination of water, solutes, and gases are treated in part II.

There are chapters on the vacuolated cell as an osmotic system, soils, absorption of water and solutes by roots, transpiration, conduction of water, conduction of solutes, and gaseous exchanges between plants and the outside air. Part III considers nutrition and metabolism. There are chapters on photosynthesis, respiration, and metabolism, in which are discussed the general metabolic processes occurring in plant tissues, including the metabolism of the fats and proteins. It would seem better to emphasize the importance of fats and proteins by discussing the synthesis and the other metabolic features of these compounds in separate chapters, or at least in a prominent subsection of a chapter. Growth and plant movements are treated in part IV. The book contains three appendixes, two of which present the more advanced material on organic compounds and physical chemistry, amplifying considerably various portions of the text proper. Appendix III is comprised of 163 citations, which are referred to by number throughout the text. In connection with the discussions of most of the plant processes, there are descriptions of experiments that may be tried in measuring or demonstrating the process. Usually there is a figure of the apparatus to be used.

In a few places in the book the author fails to emphasize recent viewpoints. For example, the absorption of salts by the plant is described as a physical process in which the particles diffuse from a region of greater concentration in the soil solution to one of less in the plant. While this may be true in some cases, there is certainly enough recent work, especially in America, to show that in many cases the salts enter against the gradient and that the greater concentration on the inside is an effective one and is not due to the salts being adsorbed or tied up in some way with the cell contents. The reviewer noticed one or two obvious errors. For example, it is stated that cane sugar is laevo-rotatory, and that the mixture of the products of hydrolysis is dextro-rotatory, since glucose is dextro-rotatory to a greater extent than fructose is laevo-rotatory.

On the whole the book is a well balanced, thorough treatment of the principal physiological processes occurring in the green plant. It should be of great interest and help, not only to the general botanist and plant physiologist, but to workers in the various applied fields such as horticulture and agronomy.— S. V. EATON.

An Illustrated Manual of Pacific Coast Trees. By HOWARD E. McMINN and EVELYN MAINO. Berkeley: University of California Press, 1935. Pp. xi+409. figs. 415.

This book should prove entirely usable both for botanists and for those generally interested in trees. Not only are the native trees characterized, but also more than 400 introduced species.

There are few places where a greater variety of environmental conditions are likely to be encountered than in the area covered by this book—California, Oregon, Washington, and parts of British Columbia, and it is therefore to be ex-

pected that a considerable number of introduced species of trees should be found there. It is helpful to have at hand a manual which makes the identification of these reasonably certain. A number of the more common fruit trees of greatest commercial importance are mentioned, but they are not characterized in detail sufficient for identification. This omission seems unfortunate, since such trees are as botanically unknown to many as are the various more or less ornamental forms which are included. Suggestions for the possible use of both the native and introduced forms as landscape material and the conditions under which the various species may be expected to thrive add greatly to the value of the book to the amateur gardener and professional landscape architect.

The keys are as simple as can be expected, and yet serve the purpose of distinguishing the species. The drawings are clear cut, and without cumbersome detail.—E. J. KRAUS.

British Stem and Leaf Fungi (Coelomycetes). Vol. I. By W. B. GROVE. New York: Macmillan & Co., 1935. Pp. xx+488. $7.

Two groups of the fungi which infect the stems and leaves of plants stand out prominently because of their economic importance. The first of these are the rust fungi (Uredinales) and the second the Coelomycetes, a group belonging to the "Imperfect Fungi," and including the Sphaeropsidales and the Melanconiales. The first group was dealt with in British Rust Fungi (Cambridge University Press, 1913). The second group GROVE now plans to present in two volumes. The first volume has recently appeared and includes those Sphaeropsidales which have colorless spores. The second volume will contain the Sphaeropsidales with colored spores and the Melanconiales.

Volume I includes the Hyalosporae, Hyalodidymae, Hyalophragmiae, and the Scolecosporae. Most of the species here treated have been seen and examined microscopically, and the account given of each species is in the main purely morphological. A complete host index and a binomial index are given.

The need for a work dealing with this group has been apparent for a long time. In the opinion of the reviewer this volume and the forthcoming one will effectively meet the need. With the keys, indexes, and species descriptions, the identification of these fungi should be made much more certain. Interest in the group should be materially stimulated, not only in Great Britain but among the students of these fungi throughout the world.—J. M. BEAL.

On the Flora, Including Vascular Land Plants, Associated with Monograptus, in Rocks of Silurian Age, from Victoria, Australia. By W. H. LANG and ISABEL C. COOKSON. Phil. Trans. Roy. Soc. Series B. No. 517. Vol. 224. Pp. 421–449. pls. 29–32. 1935.

Until recently the oldest well known floras were those of the Middle and Lower Devonian of Aberdeenshire, Scotland, and Elberfeld, Germany. Our information on fossil land plants has been extended into the Silurian by this recent

publication, which will on this account become a milestone in the history of paleobotany. It has been assumed that extensive land floras existed in the Silurian and probably earlier, but no reliable evidence had been found of Silurian plants until the localities in Australia were discovered.

The plants are unquestionably Silurian, occurring in immediate association with Silurian graptolites. These plants are the oldest definitely known vascular land plants. The authors describe two new genera, *Baragwanathia* and *Yarravia*, besides listing fragmentary branch systems, similar to the well known Devonian genus *Hostimella*. All remains of plants belonging to *Baragwanathia* can be included in one species, *B. longifolia*. They are represented by leafy shoots, in some cases bearing sporangia; by stems with incomplete leaf bases, leaf scars, or without remains of leaves; by stems with laterally placed branches that were possibly rhizomatous; and by specimens showing the structure of the vascular system. The plant was doubtless homosporous, and the position of the sporangia and their relation to the leaf bases suggest comparison with Lycopodiales rather than with Psilophytales. On the other hand, the stellate arrangements of the stelar xylem now known for *Baragwanathia* can be closely compared with that found in *Asteroxylon*.

The new genus *Yarravia* shows unbranched axes or stalks, with a large synangial fructification which appears to have included a small number of larger linear-oval sporangia. *Yarravia* seems to have had a peculiar type of fructification, suggestive of derivation from terminal groups of large sporangia of the kind known in Psallophytales. The genus contains two species, *Y. oblonga* and *Y. subsphaerica*.—A. C. NoÉ.

Rock Garden Plants. By CLARENCE ELLIOTT. New York: Longmans, Green & Co., 1935. Pp. 328. figs. 17. $3.

To those interested in rock gardens and to students of botany this very readable book should prove interesting. In a delightful style, a considerable number of species and hybrids (chiefly alpine) are described. The author has limited his discussion to plants which he has actually known, and mostly grown. Cultural directions, preparation of sites and soils, pleasing and displeasing characteristics, are generally given in detail. Genera are listed alphabetically and under these the several species and numerous hybrids. The nomenclature follows the 1934 edition of the Kew Hand List of Rock Garden Plants.—K. C. HAMNER.

The Design of Experiments. By R. A. FISHER. London: Oliver & Boyd, 1935. Pp. xi+252.

A book on a rather new subject has recently been written by FISHER. It is concerned with the planning of experiments. Certain sections of the author's well known book, Statistical methods for research workers, are concerned with the technique of agricultural experimentation, and the present book is really an expansion of these sections. After the introductory chapter, there follow three

chapters in which the principles of designing an experiment are illustrated by a psycho-physical experiment, one of Darwin's growth rate experiments, and an agricultural experiment. Questions of design have been the most thoroughly studied in the field of agricultural experimentation, and in later chapters are given descriptions of the principal designs which have been found successful in this field. The author also shows how these designs can be applied to other fields. The main designs described are: the Latin square, the factorial design in experimentation, and compounding. Other subjects discussed are the increase in precision by concomitant measurements and the null hypotheses. The book should be of help to the worker in any field of science. If one is to read the book with the greatest profit, however, he should be fairly well acquainted with the principles of statistics, as outlined in the author's preceding work.—S. V. EATON.

The Fossil Flora of Scoresby Sound, East Greenland. Part IV. By T. M. HARRIS. Kopenhagen: C. A. Reitzel's Forlog, 1935. Pp. 176. pls. 20.

Part IV of a book on the fossil floras of Scoresby Sound has just appeared. The four volumes of the publication deal with: Cryptogams, exclusive of Lycopodiales (part I); the seed plants incertae sedis (part II); the Caytonailes and Bennettitales (part III); and Ginkgoales, Coniferales, Lycopodiales, and isolated fructifications (part IV). The fifth volume is intended to contain the stratigraphic relations and ages of the floras. The entire series represents a careful study of an important Mesozoic flora, and deals with external as well as internal characters, as far as the latter were available. The author is to be congratulated upon the successful completion of the botanical part of his work. The study of cuticles as a means of identification of fossil plants has received due consideration.—A. C. NOÉ.

American Ferns. By EDITH A. ROBERTS and JULIA R. LAWRENCE. New York: Macmillan & Co., 1935. Pp. viii+98. figs. 41. $2.50.

Descriptions of a number of species and suggestions for their wider use in landscape and general garden plantings are presented. A readily workable key of identification of a number of species as they appear throughout the year is of special interest. Suggestions are made for growing plants from spores and the times at which spores of various species may be obtained are given. The illustrations are numerous and of unusual clarity and excellence.—E. J. KRAUS.

Über die Beteiligung Kalkablagernder Pflanzen bei der Bildung Südbayerischer Tuffe. "Bibliotheca Botanica" No. 110. By J. WALLNER. Stuttgart: E. Schweizerbart'sche Verlagsbuchhandlung, 1934. Pp. 30. pls. 2. RM 14.

Some of the coral reefs throughout geologic time, especially during the Mesozoic era, were formed by limestone deposits from plants. They are found in large quantities in the Alps, and a recent investigation of the formation of such vegetable coral reefs is reported in this book. The author made his observations in

southern Bavaria, in the foothills of the Alps. He investigated the form in which recent lime-secreting plants are acting and draws his conclusions from the activity of such plants during geologic time.—A. C. NOÉ.

Le Pansoma et la Géométrie de l'Energie. By A. C. LÉEMANN. Geneva: Georg and Company, 1935. Pp. 257.

Recent advances in physics and mathematics in the interpretation of matter, energy, and space will no doubt exert a profound influence upon philosophic thought. Those who are seeking some unifying principle in the diverse phenomena of nature, and those who are trying to interpret the complexities of our universe on some simple fundamental basis, will naturally turn to the newer units of matter and energy, and to the new space geometry as a point of departure. An attempt at such a simple explanation of a great range of phenomena has been made by DR. A. C. LÉEMANN of the University of Geneva, who derives the basis of his philosophy from the quantization of energy developed by PLANCK, and from the space geometry of EINSTEIN. The important step forward taken by LÉEMANN is essentially the geometrization of the quantum. No one has thought of the quantum as having three-dimensional space relations. But LÉEMANN argues from the identity of matter and energy, that if matter, one form of energy, is conceived to be three-dimensional, why should not the quantum itself be a three-dimensional energy unit? This three-dimensional unit he calls the *pansome.* It is indestructible, indivisible, conceived to be bounded sharply by pansomic surfaces, has an absolute and constant energy content, and has the power to manifest but one fundamental type of variability, that of changing its volume with its environment. Using this geometrized unit, LÉEMANN attempts to show how it is possible to give simple explanations of a great variety of natural phenomena. The fields of application will be briefly stated. He first takes up fundamental physical phenomena, energy, protons and electrons, temperature, ether, inertia, electricity, magnetism, radiations, etc. Then he passes to problems of cosmology, such as expansion of the universe, nebulae, creation of matter, conditions in the interior of stars, origin of stellar energy, etc. A little farther on he is considering affinity and valence, crystalline structure, properties of solution, adsorption, colloidal state, etc. From here it is but a step to vital phenomena, and he considers such familiar biological matters as karyokinesis, organogenesis, genetics, mutation, and evolution! Toward the close of the work, physiological processes come into the purview. Nerve conduction, muscular contraction, and the paratonic responses, both vectorial (taxies and tropisms) and non-vectorial (nasties, etc.) are considered. The last section enters the field of psychology and mental aberrations.

The author says that pansomism, in its essence, is only a kind of general psychology, which makes possible the interpretation of the marvellous harmony of nature in very simple but adequate terms. The reader may be disappointed at times because of the brevity of treatment; for although the work is extensive,

each section is rather short. On the other hand, this brevity of treatment of each topic makes it possible to stop frequently to meditate upon the applications of pansomism without too great wilderness of details. One should approach the work with open mind, and with a degree of skepticism in order most fully to enjoy the argument.—C. A. SHULL.

The Species of Tradescantia Indigenous to the United States. By EDGAR ANDER-SON and ROBERT E. WOODSON. Jamaica Plain, Mass.: Arnold Arboretum, 1935. Pp. 132. pls. 12. $2.25.

During recent years the genus *Tradescantia* has been the object of much sus-tained investigation from the viewpoints of taxonomy, physiology, cytology, and genetics. And since the combination of cytology and genetics for the study of certain evolutionary processes has developed, *Tradescantia* has lent itself ad-mirably as a subject for such work. This is not surprising, in view of the ease with which it can be grown from seeds or transplants, its ability to withstand varied environmental conditions, the long blooming period and ease of artificial pollination, and also because it has large chromosomes, and both diploid and tetraploid species and varieties occur in nature. Yet fundamental conclusions concerning the evolutionary importance of the cyto-genetic findings are not pos-sible until they can be viewed against a background of intensive morphological and taxonomical research.

With these multiple needs in mind, the authors have made a serious effort to revise the native species of *Tradescantia* occurring north of Mexico. They have restricted the revision to the species indigenous to the United States for several reasons. This group of species, which are somewhat frequent in cultivation and available in the field, have been used, in the main, for most of the recent cytologi-cal and genetical investigations. From the taxonomic point of view this group, in which *T. virginiana* L. must be considered the type species, constitutes the typical element; and also morphologically the species indigenous to the United States with two exceptions fall into distinct groups of affinity which are limited in distribution to the region chosen. The species native to Mexico, the Antilles, and Central and South America are so little known that it was deemed wise to exclude them from this presentation.

Included are the taxonomic history, gross morphology, speciation, hybridiza-tion, study material, taxonomy, and an index. Under taxonomy there are a key to the species, descriptions, citations of synonyms and specimens, excluded spe-cies, and a list of exsiccatae. Twelve plates show various morphological and taxonomic characters of representative species.

This monograph will doubtless prove to be of considerable interest and value, not only to those who are engaged in research with the members of this genus, but to those also who are working on similar problems with other groups of organisms.—J. M. BEAL.

Problems in Soil Microbiology. By D. W. CUTLER and LETTICE M. CRUMP. London: Longmans, Green & Co., 1935. Pp. vii+104. $3.20.

The lectures on soil microbiology which were delivered by D. W. CRUMP on the Aberystwyth Foundation during the 1934–35 session have been modified and published as a monograph by CUTLER and CRUMP in the Rothamsted Agricultural Science Series. They record the results of work done in the department of general microbiology of the Rothamsted Experimental Station. The lectures present the soil as a medium to which the microbiological population has become adapted by selection through long ages, until now the soil is an "eminently suitable home" for the micro flora and fauna which inhabit it. The population is unspecialized to the extent that almost any decomposable substance will be attacked and used in the general economy of the population. The interrelations of the various types of organisms, the manner in which they influence one another's activity, form an important part of the work. The seven chapter titles indicate the scope of the monograph: The suitability of the soil for microorganisms, the bacterial population under field conditions, the relation of bacteria to nitrite, carbon dioxide production by soil, the growth of protozoa in pure culture, the behavior of protozoa in soil, and the interaction among the soil organisms. The work is a welcome addition to the popular agricultural monographs coming from the Rothamsted laboratories.—C. A. SHULL.

Praktikum der Zell- und Gewebephysiologie der Pflanze. By SIEGFRIED STRUGGER. Berlin: Gebrüder Borntraeger, 1935. Pp. xi+181. RM 8.50.

Laboratory Plant Physiology. By B. S. MEYER and D. B. ANDERSON. Ann Arbor, Michigan: Edwards Brothers, 1935. $1.75.

Two laboratory manuals of plant physiology have been published during recent months. One is by STRUGGER and the other by MEYER and ANDERSON. STRUGGER's work is devoted to general cellular physiology, and presents 94 experiments, arranged in eleven topical sections.

The manual by MEYER and ANDERSON is a general work for classes in plant physiology. It is interleaved to permit taking of notes on observations. There are 132 experiments, arranged in eighteen sections with logical sequence. Both manuals should prove valuable in different types of courses.—C. A. SHULL.

Chronica Botanica. Vol. I. Edited by FR. VERDOORN. Leiden, Holland, 1935. Pp. 447.

A useful yearly publication, Chronica Botanica, made its first appearance this spring. The bulk of the volume is devoted to a review of all botanical departments in universities, gardens, museums, national societies, government bureaus, colonial departments of botany and agriculture; in short, to all local activities of a botanical character. The reports are in any of the five languages: English, German, French, Spanish, and Italian.

It is to be regretted that the questionnaire for the Chronica Botanica was not answered by a number of botany departments in large universities, especially in the United States; therefore the space given to individual institutions does not always correspond to the true importance of the same.—A. C. Noé.

Annual Review of Biochemistry. Stanford University Press, 1935. Pp. viii+639. $5.00.

Several years ago the Stanford University Press began the issue of an annual volume devoted to the recent advances in biochemistry. The fourth volume has just been issued. It contains 27 reviews, many in the fields of animal metabolism, a few in the fields of general or plant physiology. Among the latter may be mentioned the sections on permeability, biological oxidations and reductions, chemistry of the carbohydrates and glycosides, vitamins, plant pigments, the alkaloids, the mineral nutrition of plants, growth substances in plants, and the chemistry of bacteria. It is not possible for the reviewers to take into consideration all the literature produced in the past year or two in any of these fields; they must select the important facts from among the less important contributions. The writer feels that the responsible reviewers have been unusually successful in producing well written reviews that faithfully reflect the progress of biochemical science year by year. The series constitutes a remarkable record of the history of biochemistry during the period covered, and its importance will be increasingly evident as the series lengthens.

The editors of the series, J. MURRAY LUCK, C. L. ALSBERG, D. R. HOAGLAND, and C. L. A. SCHMIDT, deserve great credit for the success of this venture into the summarizing field. The Annual Review has become indispensable to physiologists who want to keep abreast of current thought and oriented to the direction of the main advances. It is gratifying that the series has met with the approval of workers, and that its successful continuation is assured.—C. A. SHULL.

Flora of Iceland and the Faeroes. By C. H. OSTENFELD and JOHS. GRÖNTVED. Copenhagen: Levin and Munksgaard, 1934. Pp. xxiv+195. maps 2.

The late Professor C. H. OSTENFELD had for several years, prior to his death in 1931, made preparations for a Flora of Iceland and the Faeroes, completing the text as far as the Leguminosae. The remainder of the text, including keys, glossary, and index, has since then been completed by JOHS. GRÖNTVED, and the whole issued as a handy volume of little more than pocket size. The text is in English. The style is simple and direct, making a special appeal "as a field-book for amateurs and botanists" who might wish to become acquainted with the flora of these islands. A large number of genera and even many species common in continental North America and known to most of our American students are represented. A special attempt at completeness in listing vernacular names is evident, the scientific names being regularly accompanied with the Icelandic or Faeroesian equivalents. Altogether, the book seems likely to prove exceptionally

usable, unless perhaps in the case of certain genera (for example, *Taraxacum*, *Hieracium*) where a number of apparently dubious specific segregations have been admitted.—E. E. SHERFF.

Les zoocécidies des plantes de l'Amérique du Sud et de l'Amérique Centrale. By C. HOUARD. Paris: Hermann & Co., 1933. Pp. 519. figs. 1027. map 1.

An important recent work on galls is the volume by HOUARD, of the University of Strasburg. It deals with the insect and other animal galls known so far for Central and South American plants. The volume is largely in the nature of a compendium for reference.

Only three pages are needed for the galls peculiar to the vascular cryptogams, as compared with about 433 pages for the phanerogams. The text is arranged in botanical sequence, a great convenience to users, who are primarily botanists. Each kind of gall or gall effect is described in detail and in numerous cases illustrated with a simple but instructive figure. General facts of classificatory significance are largely systematized and represented with suitable abbreviations. Thus *acrocécidie* (gall terminal) is indicated by Acrc. as distinguished from Plrc. for *pleurocécidie* (gall lateral). Whether the gall deforms the fruit, capitulum, inflorescence, flower, tip of stem, bud, or other part of the plant is shown by Ac.fr., Ac.cp., Ac.inf., Ac.fl., Ac.ti., etc. Abbreviations such as M.C. and M.T. indicate whether the metamorphosis of the animal organism concerned occurs in the gall or in the earth.

An extensive bibliographic index accompanies the work and two other indexes, one to the plants and one to the causal animal organisms, are given.—E. E. SHERFF.

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A CYTOLOGICAL MONOGRAPH OF THE AMERICAN SPECIES OF TRADESCANTIA

EDGAR ANDERSON AND KARL SAX

(WITH THIRTY-TWO FIGURES)

Introduction

In *Tradescantia*, as in all genera, there are certain special features of its evolutionary processes which are peculiar to itself and to its immediate relatives; certain general ones which are more fundamental. To sift the special from the general and to examine both, and to measure the evolutionary processes taking place in the American species at the present time, have been and still remain the objective of a comprehensive program of cooperative research. A taxonomic monograph of the American species has recently appeared (3), and a preliminary discussion (1) and isolated technical papers (2, 14, 19, 20, 21) dealing with certain phases of the problem have already been published. The following pages bring together a general cytological survey of the problem and a discussion of the bearing of this evidence.

Chromosome number and morphology in the Commelinaceae

An evaluation of the phylogenetic importance of the various evolutionary processes in *Tradescantia* will have greater significance if preceded by a cytological survey of the Commelinaceae as a whole. Such a survey should yield useful evidence as to the importance of structural changes in the chromosomes. The differentiation of species or even of genera may be dependent upon gene mutation with no

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apparent change in chromosome structure or morphology; but in
many cases differentiation is associated with structural and numeri-
cal changes in the chromosomes. These changes may include (1)
autopolyploidy, the duplication of similar genoms; (2) aneuploidy,
the duplication of only a few chromosomes of the genom; (3) seg-
mental interchange between non-homologous chromosomes which
may change the morphology of the genom; (4) fragmentation and
loss of chromosome segments in polyploids; (5) inversion or trans-
location of segments; and (6) changes in chromosome size. Some of
these numerical and structural changes may be directly effective in
differentiation, but their chief function is providing the initial isola-
tion permitting independent development of genic changes. Another
important factor in differentiation is (7) allopolyploidy, the union
and duplication of unlike genoms. This process involves a simulta-
neous change in genetic constitution and chromosome number.

The Commelinaceae are especially favorable material for a cyto-
logical study of the differentiation of genera and species. Many of
the genera have been studied in some detail by DARLINGTON (6), who
reviews the rather meager literature on the cytology of this group of
plants. Unfortunately the family is much in need of comprehensive
monographic treatment. The most recent attempt (5), while a great
advance, is admittedly incomplete. A general discussion of evolu-
tionary trends within the family, in the light of the cytological evi-
dence, will scarcely be possible until the delimitation of the genera
and their logical classification have been more adequately considered.

We have made a cytological study of nine genera and thirty-two
species. To these can be added the information obtained by DAR-
LINGTON, bringing the totals to thirteen genera and about thirty-
seven species. The analysis of the genus *Tradescantia* has been done
in considerable detail, and all the species native to the United States
have been examined, with the exception of *T. wrightii* and *T. pine-
torum*.

The data on chromosome number and morphology have been ob-
tained from aceto-carmine smears of microsporocytes and developing
microspores. The microspore division is especially favorable for
study, because the metaphase plate is relatively flat and the single
genom is present. The chiasma frequency at meiosis and pollen fer-

tility have been determined for several species, for artificial F_1 hy-
. brids, and for plants from hybrid colonies.

The common Tradescantias of the United States belong to a
group of species all closely related to *T. virginiana*. The diploid
forms have six pairs of large chromosomes, while the tetraploids
have twelve pairs of chromosomes. The following species are
known only as diploids: *T. gigantea*, *T. humilis*, *T. edwardsiana*, *T.
paludosa*, *T. ernestiana*, and *T. hirsuticaulis*. *T. bracteata* is usually
diploid, but a triploid form has been found. Both diploid and tetra-
ploid forms have been found in *T. hirsutiflora*, *T. occidentalis*, and *T.
canaliculata*. *T. subaspera* var. *typica*, *T. subaspera* var. *montana*, *T.
roseolens*, *T. virginiana*, *T. longipes*, *T. ozarkana*, and *T. tharpii* have
been found only as tetraploids. It is probable that certain species
listed as only diploid or only tetraploid may be found to contain both
types as more individuals are studied. The diploid and tetraploid
forms within a single species are very similar in morphological char-
acters, and cannot be distinguished without cytological analysis.

The chromosomes of the typical Tradescantias have median or
submedian fiber constrictions (figs. 1, 3, 24). There is no consistent
difference in the chromosome size of different diploid species, or with-
in the tetraploid forms, but the tetraploids in general have somewhat
shorter chromosomes than have the diploids.

Segmental interchange has been found in occasional plants of four
diploid species, *T. edwardsiana*, *T. humilis*, *T. canaliculata*, and *T.
gigantea*. Reciprocal translocations had occurred between two non-
homologous chromosomes. The interchanges were approximately
equal so that the microspore chromosomes were indistinguishable
from those of normal plants, but at meiosis the interchange plants
form a ring or chain of four chromosomes and four bivalents (fig. 20).
The segregation of the chromosomes in the interchange ring results
in about 50 per cent pollen sterility (19). Segmental interchange was
also found in certain tetraploid species, and rings of six or eight
chromosomes were observed; but the cytological complexity of the
tetraploid prevents ready analysis of interchanges by cytological ob-
servation through the failure of any consistent behavior of inter-
change chromosomes.

Chromosome fragments have been found in both diploids and

tetraploids. These are usually small chromosome segments with a terminal or subterminal fiber attachment point (fig. 21). They are rather regular in behavior in both mitotic and meiotic cells and are transmitted by both egg cell and pollen grains. The number of frag-
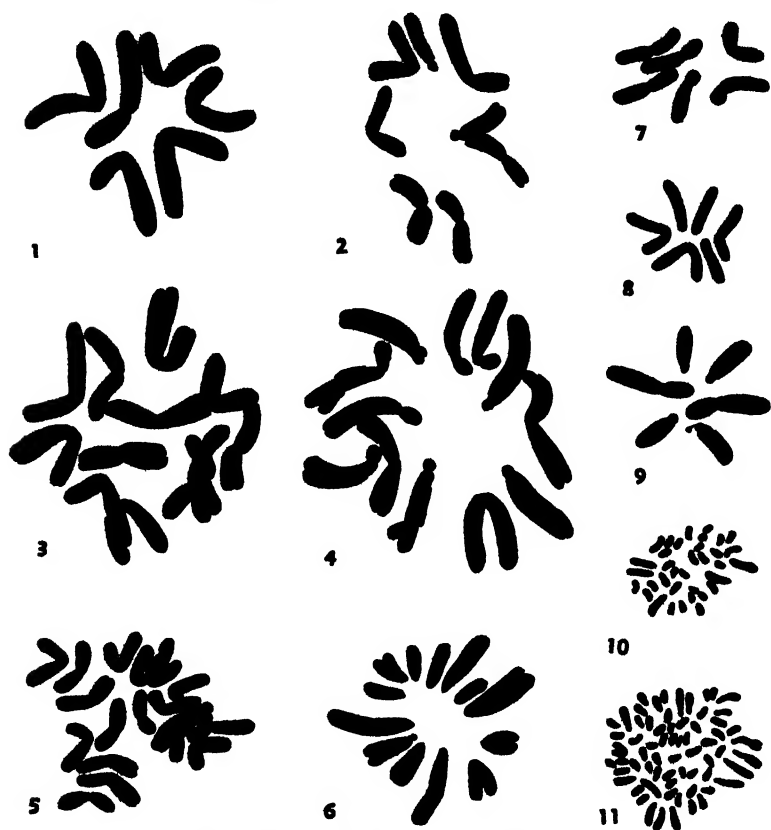
FIGS. 1–11.—Chromosomes of microspore nuclei in species and genera of Commelinaceae. From aceto-carmine smears: Fig. 1, *Tradescantia humilis;* fig. 2, *Tradescantia* sp. "Victoria"; fig. 3, *T. canaliculata;* fig. 4, *T. rosea;* fig. 5, *Setcreasea brevifolia;* fig. 6, *T. micrantha;* fig. 7, *Rhoeo discolor;* fig. 8, *Spironema fragrans;* fig. 9, *Callisia repens;* fig. 10, *T. geniculata;* fig. 11, *T.* sp. "bench." ×1200.

ments may vary from one to a dozen or more in diploid plants with little or no change in the appearance or the fertility of the plant. While particularly common in *T. paludosa*, they have been found in all the other species which have been extensively examined. A de-

tailed study of the behavior of the small fragments has been made
by DR. T. W. WHITAKER (in press). Much larger fragment chro-
mosomes have occasionally been found, particularly among plants
from localities where specific hybridization was suspected.

The genoms of the tropical species of *Tradescantia* may differ from
those of the Virginiana group in number, size, and morphology of the
chromosomes. *Tradescantia* "Victoria" is an unidentified Mexican
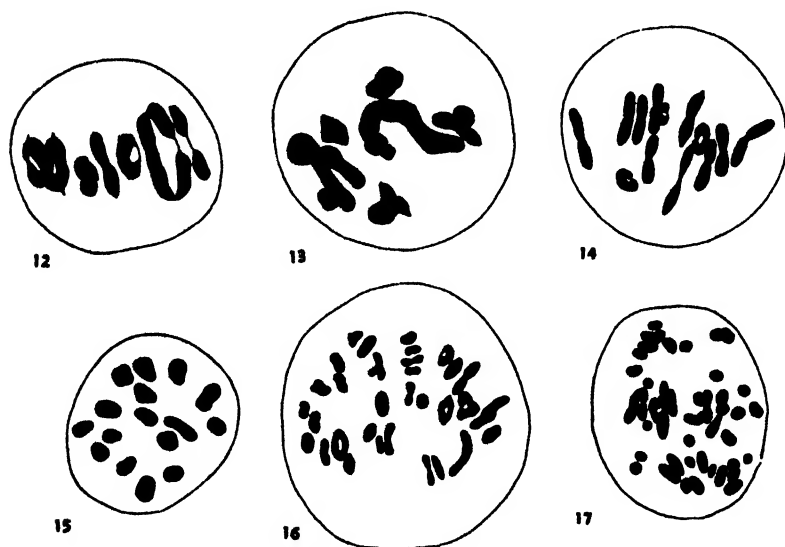
FIGS. 12–17.—Meiotic divisions in genera of Commelinaceae: Fig. 12, *Tradescantia*
sp. "Victoria," four bivalents and two quadrivalents; fig. 13, *Campelia zanonia*, four
bivalents and two chains of four chromosomes each; fig. 14, *C. anomala*, thirteen
bivalents; fig. 15, *Tradescantia geniculata*, sixteen bivalents; fig. 16, *Tinantia fugax*,
about thirty-two bivalents; fig. 17, *Commelina benghalensis* with about six bivalents and
thirty-six univalents ×1000.

species allied to *T. geniculata*. The haploid chromosome number is
eight, including six chromosomes with median fiber constrictions and
two with subterminal constrictions (fig. 2). At meiosis there are
usually two ring bivalents and two quadrivalent rings or chains of
four chromosomes each (fig. 12).

There are several reasons for believing that this plant is an aneu-
ploid with two duplicate chromosomes. In species with the basic
number of chromosomes, such as the diploid Tradescantias, the

microspore nucleus does not divide unless it contains a complete genom. In Victoria the microspore nucleus may divide with only seven chromosomes, indicating that the absent chromosome has a duplicate homologue. If the quadrivalents were segmental interchange rings, random distribution would produce about 75 per cent sterility, but 94 per cent of the pollen is good. Therefore, the rings of four chromosomes must be quadrivalents, each with four homologous chromosomes. The frequency of bivalent formation also supports the assumption that these rings are quadrivalents. In normal tetraploids about one-third of the chromosomes pair as bivalents at meiosis, while in segmental interchange plants the interchange chromosomes seldom form bivalents. We may conclude then that Victoria is an aneuploid individual with four pairs of chromosomes and two quadrivalents.

Tradescantia rosea (*Cuthbertia rosea* Sm.) of southeastern United States has twelve pairs of large chromosomes, four of which resemble the chromosomes of species allied to *T. virginiana*, while eight have subterminal fiber constrictions (fig. 4). The reduction divisions are regular and the pollen fertility is normal.

Tradescantia micrantha of the Gulf Coast has thirteen pairs of chromosomes all of which have terminal or subterminal fiber attachment points. Four of the chromosomes are about as large as those of *T. rosea* but the others are much shorter (fig. 6).

There are sixteen pairs of chromosomes in *T. geniculata* and only bivalents are formed at meiosis (fig. 15). Four of the chromosomes have more or less median fiber constriction points while twelve are terminal or subterminal. According to DARLINGTON, *T. navicularis* also has sixteen pairs of chromosomes, but in this species all chromosomes have subterminal fiber constrictions. In both species the chromosomes are relatively large.

DARLINGTON found thirty pairs of small chromosomes in *T. fluminensis*. We have found about thirty-five pairs of small chromosomes in a species allied to *T. geniculata* (fig. 10), and about sixty-five pairs of chromosomes in an undetermined tropical species (fig. 11). In all these species with small chromosomes the fiber constrictions are usually terminal or subterminal.

The monotypic genus *Rhoeo* has twelve chromosomes in somatic

cells. At meiosis these twelve chromosomes are attached end to end to form a ring or one or more chains of chromosomes. In this segmental interchange ring no two chromosomes are completely homologous and the order of the chromosomes is always the same (fig. 23). The behavior of these chromosomes has been described in considerable detail (15, 16). Four of the twelve chromosomes have subterminal fiber constrictions while the other eight are more or less median (fig. 7). The high frequency of irregular distribution of chromosomes at meiosis results in about 80 per cent pollen sterility, but seeds are produced and the progeny breed true to type.

Spironema fragrans (fig. 8) and *Callisia repens* (fig. 9) have genoms with two median or submedian chromosomes and four with subterminal fiber attachments. Although the genoms are somewhat similar in chromosome morphology and chromosome size, the differences in plant size are about as extreme as can be found in the Commelinaceae. Aside from mere size, the genera are morphologically similar and are usually classified by taxonomists as being very closely related (5).

The chromosomes of *Setcreasea* resemble those of the Virginiana group of *Tradescantia* in respect to the location of the fiber constrictions, but they are somewhat smaller in size. DARLINGTON found twelve pairs of chromosomes in *Setcreasea brevifolia* (= *Treleasia brevifolia* Rose) while we find eighteen pairs of chromosomes in this species (fig. 5). At meiosis about half of the chromosomes are paired as hexavalents while the others usually form bivalents and quadrivalents. About 80 per cent of the pollen is good.

Zebrina pendula has twelve pairs of large chromosomes, ten of which have subterminal fiber attachments.

The monotypic genus *Campelia zanonia* has a basic number of eight and all the chromosomes are large. At meiosis there are four bivalents and two chains of four chromosomes each (fig. 13). These chains appear to be the result of segmental interchange rather than of chromosome duplication, although we have insufficient data to determine this point conclusively.

The haploid chromosome number is twenty-eight in *Commelina nudiflora* and about forty-five in *C. coelestis* (6). DARLINGTON found thirty-four chromosomes in *Commelina benghalensis*, but in the form

we have used there are about six bivalents and thirty-six univalents (fig. 17). In this plant there is complete pollen sterility. The fact that there are six bivalents indicates that there is no general suppression of meiosis and yet there are about thirty-six univalents. Such behavior would seem to indicate that the univalents include unlike genoms and that allopolyploidy is a factor in species differentiation in the Commelinaceae.

DARLINGTON has obtained chromosome counts of *Coleotrype natalensis* (n = 18), *Dischorisandra thyrsiflora* (n = 19), and *Tinantia fugax* (n = 32). Of these genera we have examined only *T. fugax* and find about thirty-two bivalents at meiosis (fig. 16). There are about forty bivalents in *Palisota bracteata*.

A list of the various genera and many of the species is given in table I. The haploid chromosome number (n) is given, together with the number of chromosomes with more or less median fiber constrictions and those with subterminal constrictions, and the approximate relative sizes—large (l), medium (m), or small (s). The counts were made by DARLINGTON (D) or by the junior writer (S). We have not included the individual species in the Virginiana group of Tradescantias because their genoms are morphologically similar.

GENERIC AND SPECIFIC DIFFERENTIATION IN THE COMMELINACEAE

All of the known factors which may be associated with taxonomic differentiation seem to be involved in the Commelinaceae. The differentiation of genera and species may be associated with gene mutation, autopolyploidy, allopolyploidy, aneuploidy, segmental interchange, or loss of chromosome segments.

The diploid species related to *T. virginiana* are apparently differentiated only by gene mutation. The genoms are similar in respect to chromosome number and chromosome morphology, and a cytological analysis of species hybrids shows that the genoms are similar in structure. Since these species are interfertile, isolation is a necessary factor in species differentiation. This isolation seems to have been effected by differences in ecological adaptation, as will be discussed later in this paper.

Autopolyploidy is also found in the Virginiana Tradescantias. Autopolyploidy in this genus has no direct relation to speciation

since tetraploidy induces little or no change in morphological characters in *Tradescantia*. Tetraploidy, however, may serve to isolate a new variant, since even intraspecific hybrids between tetraploids and diploids are about 50 per cent sterile. The tetraploids have a

TABLE I

CHROMOSOME NUMBER AND MORPHOLOGY IN THE COMMELINACEAE

GENUS AND SPECIES	CHROMO-SOME NO.	FIBER CONSTRICTION		SIZE	AUTHOR-ITY
		MEDIAN	SUBTER-MINAL		
Tradescantia (Virginiana species)....	6	6	....	l	S
T. sp. "Victoria"..................	8	6	2	m	S
T. (Virginiana species)..............	12	12	....	l	D, S
T. rosea Vent................	12	5	7	l	S
T. micrantha Torr..................	13	....	13	l	S
T. geniculata Jacq.................	16	4	12	m	D, S
T. navicularis Ortgies..............	16		16	l	D
T. fluminensis Vell.................	30	....	....	s	D
T. "geniculata"....................	35	....	....	s	S
T. albiflora Kth...................	36	....	.... ..	s	S
T. sp. "bench".....................	65	....		s	S
Rhoeo discolor Hance..............	6	4	2	m	D, S
Spironema fragrans Lindl...........	6	2	4	m	D, S
Callisia repens L..................	6	2	4	m	S
Campelia zanonia H. B. K..........	8	6	2	l	S
C. nudiflora L....................	28	....	....	s	D
C. benghalensis L.	24-34	....	. .....	s	D, S
C. coelestis Willd.......	45	....	.... ..	s	D
Setcreasea brevifolia (Rose) K. Schum. et Syd.......	12-18	12-18	....	m	D, S
Zebrina pendula Schnitzel........ .	12	2	10	l	D
Commelinantia anomala (Torr.) Tharp...................	13	3	10	m	S
Cyanotis somaliensis C. B. Clarke...	14	3	11	Very s	D
Coeleotrype natalensis C. B. Clarke..	18	4	14	s	D
Dischorisandra thyrsiflora Mikar....	19	....	....	m	D
Tinantia fugax Scheidw............	32	....	....	s	D, S
Palisota bractiosa C. B. Clarke......	40	....	....	m	S

longer blooming period than the diploids and have a higher survival value, as is shown by their greater distribution. The species known only as tetraploids may have been differentiated from their diploid ancestors by gene mutation, or the diploid ancestors may have been eliminated by natural selection, leaving only the tetraploid forms of these species.

The duplication of only a few of the chromosomes of the genom,

or aneuploidy, may be effective in speciation by producing an unbalanced relation between genes, or it may serve indirectly by effecting partial or complete isolation of the new variant. The species listed as Victoria is an aneuploid type with two duplicate chromosomes in the genom. A number of other aneuploids have been found in *Tradescantia* and other genera.

Changes in chromosome morphology can be effected in both diploid and polyploid species by segmental interchange or translocation of chromosome segments. Segmental interchange has been found in several diploid species of *Tradescantia*, but there is no evidence that it has been a factor in the differentiation of these species. In the polyploid species the differences in chromosome morphology may be attributed to segmental interchange or loss of chromosome segments. Seven of the twelve chromosomes of *T. rosea* have subterminal fibers and all of the thirteen chromosomes in *T. micrantha* have subterminal fiber attachments, while in the Virginiana tetraploids all chromosomes have approximately median fiber constrictions. In a polyploid, chromosome segments may be lost so long as the duplicate segment remains, so that many of the chromosomes might lose an entire arm as is possibly the case in *T. micrantha* and other species with heterobrachial chromosomes.

In polyploid species which have only bivalents formed at meiosis, as in *T. geniculata* and in *Commelinantia anomala*, allopolyploidy may be involved. Such an interpretation is also in accord with the behavior of univalents in *Commelina benghalensis*. Segmental interchange and loss of segments in a polyploid would tend to produce the same result, however, although it seems improbable that multivalent chromosome association would be completely suppressed as it is in many of these polyploid species.

There is clear evidence of structural differences of the chromosomes in the different genera of the Commelinaceae. The diploid Tradescantias have six chromosomes, all of which have approximately median fiber attachments; in *Rhoeo discolor* two of the six chromosomes are somewhat subterminal, while in *Spironema fragrans* and *Callisia repens* four of the six chromosomes have subterminal fiber attachments. These differences appear to have originated by segmental interchange as is clearly the case in *Rhoeo*.

In the Commelinaceae the chromosomes of different genera, or even of different species, may differ greatly in size. It seems improbable that the number of essential genes varies greatly in different diploid genera. The width of the chromosomes is rather closely associated with their length. The length of the meiotic spireme in *Rhoeo* is as great as it is in the diploid Tradescantias, but the meiotic metaphase and microspore metaphase chromosomes of *Tradescantia* are almost twice as long as those of *Rhoeo*. The differences in chromosome size may be caused by the amount of chromatin produced by the gene string during the chromosome cycle. A small amount of chromatin would permit a closer association of chromomeres and a more compact coiling of the gene string, resulting in metaphase chromosomes decreased in both length and width. Within a species the chromosomes have the same diameter regardless of their length, and even the fragment chromosomes in *Tradescantia* form a chromonema coil or partial coil which is as wide as the gyres of chromonemata in normal chromosomes.

CHROMOSOME PAIRING IN TRADESCANTIA SPECIES

The pairing of homologous chromosomes at meiosis has been studied in considerable detail. This study·was made in order to obtain additional information on the mechanism of meiosis and to aid in the analysis of species hybrids.

The prophase stages of meiosis are difficult to follow, but there seems to be complete pairing of the chromosomes at pachytene. We have not been able to make any accurate study of early chiasma formation. Occasionally diplotene or early diakinesis chromosomes seem to show a number of interstitial chiasmata, but these may be overlaps or temporary adhesions. At metaphase the chiasmata are largely terminal or subterminal.

The study of chromosome structure and behavior at metaphase has permitted an interpretation of the earlier stages which cannot be determined directly. The interpretation is based on the behavior of small fragments, location of chiasmata, and the coiling of the chromatids at metaphase and anaphase.

WHITAKER (in press) has found that small fragments with terminal fiber attachments pair almost as regularly as the normal biva-

lents which are about seven times as long. There is good evidence that these fragment chromosomes are duplicates of median segments of the normal genom. If chiasma formation is at random one might expect a fragment to be paired near the spindle fiber region of a normal chromosome, as MATHER (12) has found in *Lilium*. But in the diploid plants of *T. paludosa* examined, fragments were never found paired with a major chromosome.

The chiasmata are almost always terminal in tetraploid Tradescantias and about 80 per cent are terminal in most diploid species. The nature of the coiled chromatids indicates that the prevalence of terminal chiasmata is not due to the terminalization of interstitial chiasmata. In plants with terminal chiasmata, such as *Tradescantia*, *Rhoeo*, *Secale*, and *Gasteria*, the direction of coiling of the chromatids at the first meiotic anaphase is seldom reversed (17), but in *Trillium* (8), *Lilium* (9), and *Vicia* (SAX unpublished), where interstitial chiasmata are frequent, the direction of coiling of chromatids is reversed rather frequently. Evidently the direction of coiling on either side of a chiasma is at random, so that the changes in direction of coiling of anaphase chromatids should be approximately twice the chiasma frequency at metaphase.

The failure of pairing between the small fragments and major chromosomes, the prevalence of terminal chiasmata, and the rare reversal of coiling in *Tradescantia* chromosomes can be explained on the assumption that the distal ends of chromosomes pair early enough to insure chiasma formation or at least to insure an intimate association of the distal ends of homologous chromosomes; but by the time the median regions are paired chiasma formation is inhibited. The pairing of normal chromosomes at metaphase is largely dependent on the terminal association of the bivalents. In the fragment chromosomes, complete association occurs early enough to insure regular pairing at meiosis, since the effective pairing length is almost as great as in the major chromosomes. This interpretation of chromosome pairing in *Tradescantia* explains the prevalence of proximal interlocking of non-homologous bivalents, and invalidates the use of the data as evidence in favor of the classic theory of chiasma formation, a possibility suggested by the writers (19) and reiterated by MATHER (12).

Chiasma frequency even within a single species of *Tradescantia* may be affected by environmental conditions or, presumably, by genetic factors. Preliminary experiments have shown that when plants are subjected to sudden temperature changes the chiasma frequency is greatly reduced. A diploid plant of *T. canaliculata* was placed in a temperature chamber at 5°–10° C. for several days and then transferred to a temperature of 30°–35° C. for three days. Partial or complete asynapsis was produced by this treatment (fig. 19). Plants transferred directly from the greenhouse to the higher temperature showed no change in chiasma frequencies. Apparently rather extreme temperature changes are necessary to induce asynapsis, and there is some evidence that relative, rather than absolute, temperatures are involved. Chromosome pairing in *Rhoeo* seems to be more susceptible to temperature changes than in *Tradescantia* (17). There is some indirect evidence that temperature changes in nature may affect chromosome pairing. In certain Tradescantias, and especially in structural or genetic hybrids, the pollen fertility may be relatively high during a period of good weather, but after a few cold wet days during the summer months, the pollen fertility may decrease considerably.

The genetic factors in chiasma frequency are indicated by the consistent variation within and between species, and the frequency in species hybrids. The data obtained from five diploid species are shown in table II, and include the number of microsporocytes examined, the average chiasma frequency per bivalent, and the percentage of interlocking between non-homologous chromosomes. The data on *T. paludosa* were obtained by E. D. KING. In the same species the average chiasma frequency may vary considerably in different plants. For example, in plant 12 the chiasma frequency is 2.5 while in plant 13–11 the average chiasma frequency is 1.7 per bivalent. Each average was based on 600 cells or 3600 bivalents.

There are some consistent differences in chiasma frequencies of different species, although the variation in different plants of a given species may be considerable. The chiasma frequency per bivalent is about 2.5 in *T. gigantea* but is only 1.8 in *T. humilis*. The differences in the number of interstitial chiasmata in different species are especially clear.

The percentage of interlocking between non-homologous chromo-
somes varies greatly even in the same species. Apparently such vari-
ation is caused by minor environmental factors which do not affect
chiasma frequencies. This interlocking in *Tradescantia* and other
genera may be an important, although indirect, factor in segmental
interchange (19).

The pairing of chromosomes in the polyploid Tradescantias is of
special interest because this genus is one of the few which has multi-

TABLE II

CHROMOSOME PAIRING IN DIPLOID SPECIES

SPECIES	PLANT NO.	NO. CELLS	AVERAGE XTA PER BIVALENT	PERCENT-AGE INTER-LOCKING
T. paludosa.............	10	40	2.5	3
	11	24	2.4	7
	12	600	2.5	1
	12-1	100	2.2	1
	13-1	40	1.9	23
	13-2	40	1.9	14
	13-3	40	1.9	4
	13-4	20	2.2	0
	13-11	600	1.7	2
	14	120	2.3	27
	30	30	1.6	1
T. humilis.......	....	100	1.7	5
		20	1.8	....
T. bracteata.............	....	40	1 9	... .
T. gigantea.....	8	100	2.4	17
	9	40	2.5	15
	10	20	2.6	5
T. canaliculata...........	....	20	2.6	3

valent chromosome pairing and maintains a high degree of fertility
in nature. An autotriploid form of *T. bracteata* has been examined by
KING, and we have studied chromosome pairing in several auto-
tetraploids. The data on chromosome pairing in polyploids are
shown in table III, and include the number of cells examined, the
chiasma frequency per chromosome, pollen fertility, and the number
of univalents, bivalents, trivalents, and quadrivalents. The chiasma
frequency per chromosome is used instead of the frequency per biva-
lent since so many of the chromosomes form multivalent associations

at meiosis. If chromosomes are associated only in pairs at pachytene, the pairing length in a triploid is no greater than in a diploid, while in a tetraploid the pairing length may be twice that of the diploid (7).

In the diploid species of *Tradescantia* the chiasma frequency ranges from about 1.8 to 2.5 per bivalent, or about 0.9 to 1.3 per chromosome. In the diploid form of *T. bracteata* the chiasma frequency is about 1.0 per chromosome while in the triploid form it is 0.8. The total chiasma frequency in the triploids is greater than in the corresponding diploid, although the pairing length of the chromosomes is the same if association at pachytene is only in pairs. The

TABLE III

CHIASMA FREQUENCIES IN POLYPLOID TRADESCANTIAS

SPECIES	No. CELLS	X_TA PER CHROMO-SOME	POLLEN FERTIL-ITY	I	II	III	IV
Triploid							
T. bracteata...	100	0 8	47	63	63	537	.... .
Tetraploid							
T. virginiana.........	20	0 8	89	....	93	....	72
Ts×3.	33	0 9	85	1	174	1	110
Hillsboro (T. canalicu-lata×T. virginiana)...	17	0 8	84	4	70	4	63

greater chiasma frequency in the triploid cannot be attributed to the more rapid pairing, thereby increasing the effective pairing length, because almost all chiasmata in the triploid are terminal (99.7 per cent as compared with 84 per cent of terminal chiasmata in the diploid form). Evidence has been presented which indicates that there is little or no terminalization of chiasmata in *Tradescantia* before anaphase.

The chiasma frequency per chromosome in the tetraploids is lower than in the diploids although the pairing length per chromosome is the same, if association is only by pairs at pachytene. Almost all chiasmata are terminal in the tetraploids. The reduction in chiasma frequency in the tetraploid may be caused by competition in pairing which delays pairing and inhibits chiasma formation.

The various forms of trivalents in the triploid have been described

by KING. In the tetraploids more than half of the chromosomes are paired in quadrivalents. These are usually in the form of chains or rings (fig. 18). In most cases the chromosomes are oriented so that adjacent chromosomes in the quadrivalents pass to opposite poles, although exceptions are frequent. In *T. virginiana* we have observed various types of quadrivalents including figure 8 forms, and ring and rod figures of various forms. Interlocking between bivalents and quadrivalents is frequent (figs. 18-24).

Pollen fertility is relatively high in both diploid and tetraploid species of *Tradescantia*. In the triploids about half of the pollen is

TABLE IV

SPECIES OF TRADESCANTIA ALLIED TO T. VIRGINIANA; POLLEN STERILITY

DIPLOIDS			TETRAPLOIDS		
SPECIES	No. PLANTS	FERTILE POLLEN (%)	SPECIES	No PLANTS	FERTILE POLLEN (%)
T. canaliculata	3	90	T. canaliculata	10	77
T. occidentalis	12	94	T. occidentalis	22	89
T. edwardsiana	2	98	T. virginiana	2	89
T. humilis	3	90	T. subaspera montana	12	71
T. gigantea	14	82	T. subaspera typica	2	75

sterile, caused by irregular distribution of chromosomes. In the diploid species the pollen fertility usually exceeds 90 per cent. In the tetraploid forms the pollen fertility is somewhat lower, ranging from 70 to about 90 per cent. The pollen fertility of a number of species is shown in table IV, and two of the species include both the diploid and the tetraploid forms. The slightly greater sterility of the tetraploids is attributed to the somewhat greater irregularity in chromosome distribution at meiosis.

Hybridization

ARTIFICIAL HYBRIDS

In genera whose chromosomes are large enough to permit detailed observation, a cytological study of first generation hybrids should yield data of some phylogenetic significance. The pairing of chromo-
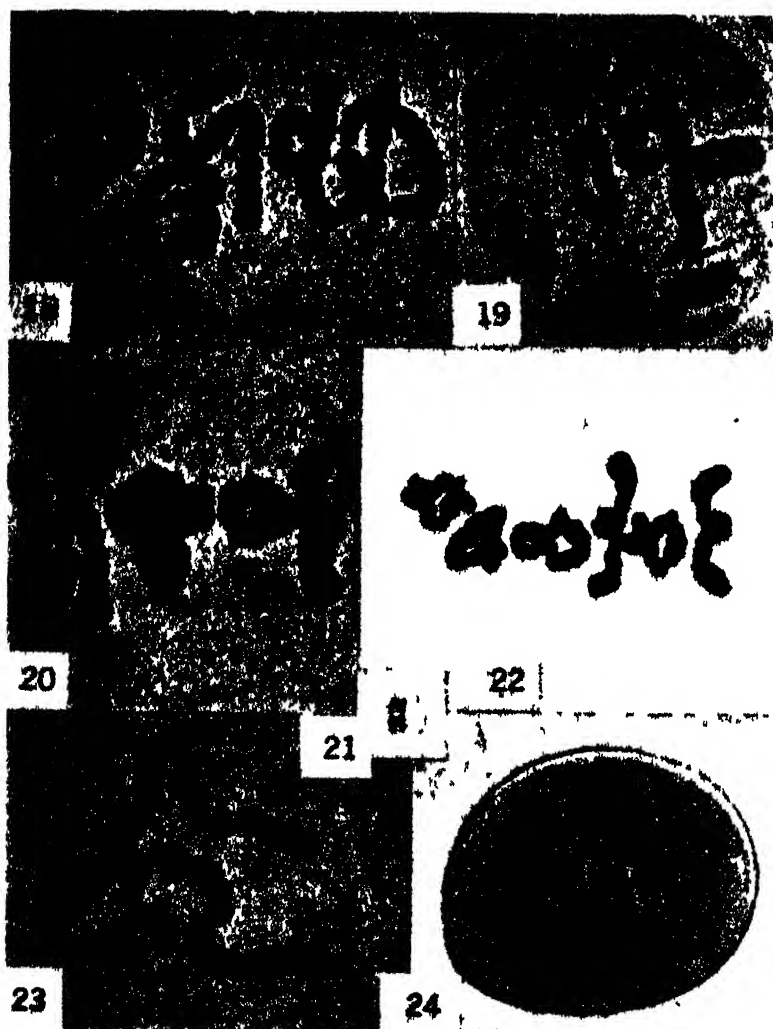
FIGS. 18–24 —Photographs from aceto-carmine smear preparations: Fig 18, first meiotic division in *Tradescantia virginiana* showing quadrivalents and bivalents. ×1200; fig. 19, partial suppression of meiosis in *T canaliculata* by abnormal temperature. ×1200; fig. 20, first meiotic division in a plant of *T. gigantea* showing segmental interchange chain of four chromosomes, interlocked ring bivalents, free ring bivalent, and rod bivalent. ×1500; fig 21, pairing of chromosome fragments at meiosis in *T. paludosa*. ×1200 (from a permanent smear preparation made by Dr T. W. WHITAKER); fig. 22, first meiotic division in F₁ hybrid of *T hirsutiflora*×*T. canaliculata* showing high frequency of interstitial chiasmata ×1200; fig. 23, segmental interchange chain of twelve chromosomes at meiosis in *Rhoeo discolor*. ×2000; fig. 24, haploid set of chromosomes at metaphase in microspore division of *T. canaliculata*. ×1200.

somes by homologous portions rather than by a generalized attraction seems to be well established as a working hypothesis, although a few exceptional cases have been described. On this hypothesis a study of metaphase configurations and an analysis of chiasma frequencies should demonstrate whether or not gross physical rearrangements of the genoms have taken place in the development of the species in question.

Most of the diploid species of *Tradescantia* are of southern origin and are not winter hardy in the northern states. It is comparatively easy, however, to grow them in the greenhouse, where they usually come into flower in February. It is difficult to emasculate the flowers in the bud without injury, but since all the species related to *T. virginiana* are self-sterile (2), they can be emasculated after the flower opens and before much pollen is shed. The flowers are pollinated immediately after emasculation and the seed ripens in about a month. An entire head is devoted to a single cross and pollinations are made on the flowers as they develop for a period of several days or longer. When the capsules begin to ripen the entire head is inclosed in cheesecloth to catch the seeds when the capsules explode. About three seeds are set per capsule and these do not germinate readily.

The hybrids produced by artificial crossing of diploid species are listed in table V, with the number of microsporocytes examined, the average chiasma frequency per bivalent, and the percentage of pollen fertility. The chromosomes of the F_1 hybrids pair about as regularly as do those of the parental species. The average chiasma frequency of *T. paludosa* is about 2.1 per bivalent and is 1.8 for *T. humilis*. In the F_1 hybrids between these species the average chiasma frequency is 1.8 to 1.9. In the case of *T. canaliculata* $\times$ *T. paludosa* the chiasma frequency of the parents is about 2.6 and 2.1 respectively, while for the F_1 it is 2.0. In the cross *T. canaliculata* $\times$ *T. gigantea* the F_1 chiasma frequency is 2.2 as compared with 2.6 and 2.5 respectively for the parental species. Somewhat similar results were obtained from the cross between *T. paludosa* and a natural hybrid between *T. humilis* and *T. canaliculata*, known in our records as "Oakhill."

In these hybrids there is, in general, a somewhat lower chiasma frequency in the F_1 than is obtained by averaging the chiasma frequencies of the parental species, and in some cases the F_1 chiasma fre-

quency is lower than that of either parent. In view of the variation of individual plants within a species, the comparisons of chiasma frequencies of parental species and of the F_1 hybrids may not be significant, although the hybrids are consistently lower than the parental averages. In general, however, the behavior of the F_1 chromosomes at meiosis would never suggest their hybrid origin. Meiotic pairing is usually regular with only occasional univalents; there is no evidence of segmental interchange rings or chains; and the distribution

TABLE V

TRADESCANTIA HYBRIDS

SPECIES	NO. CELLS	XTA PER BIVALENT	POLLEN FERTILITY (%)
Artificial hybrids			
T. paludosa×T. humilis..............	30	1.8	66
T. paludosa×T. humilis..............	100	1.9	....
T. hirsutiflora×T. canaliculata...... ..	30	2.9	73
T. canaliculata×T. paludosa..........	30	1.9	47
T. canaliculata×T. paludosa..........	100	1.9	....
T. canaliculata×T. paludosa..........	200	2.0	40
T. canaliculata×T. paludosa..........	100	2.2	....
T. canaliculata×T. gigantea..........	100	2.2	40
T. paludosa×"Oakhill"...............	30	1.6	41
Natural hybrids			
T. canaliculata×T. humilis (Oakhill)...	30	1.6	74
T. canaliculata×T. humilis (Oakhill)...	240	1.9	....
T. canaliculata×T. humilis (Oakhill)...	40	1.7	....

and behavior of chiasmata are essentially the same as in the parental species.

In one of the species hybrids, *T. hirsutiflora*×*T. canaliculata*, the F_1 chiasma frequency is relatively high and exceeds that of any of the diploid species which we have examined (fig. 21). Unfortunately we had only a single plant of *T. hirsutiflora* and did not obtain its chiasma frequency before it was discarded. The chiasma frequency of *T. canaliculata* is among the highest found—2.6 per bivalent; but for the F_1 the frequency is 2.9. It seems unlikely that the other parent would have a chiasma frequency greater than 2.9.

Although the chromosome behavior in F_1 plants shows little evidence of hybridity, the pollen sterility does show clearly that there are incompatible factors involved in these hybrids. The pollen fer-

tility of diploid species usually exceeds 90 per cent, but in the hybrids the pollen fertility ranges from 40 to 74 per cent. Even in crosses between species which are somewhat similar in morphological characters, such as *T. canaliculata* and *T. paludosa*, the pollen fertility is less than 50 per cent.

Since there is no evidence of structural changes in the chromosomes of different species, as indicated by the behavior of the chromosomes in F_1 hybrids, the differences between species must lie primarily in differences in genetic constitution. When structural changes occur in a species, as we have found in six plants, they are easily detected in the heterozygous condition by the formation of segmental interchange rings or chains of four chromosomes. It is possible that minute structural alterations would occur so that interchange rings would not be formed: but in all plants of pure species which have had a consistently low pollen fertility, we have found cytological evidence of structural changes in the chromosomes. The evidence seems to indicate that the low pollen fertility of the F_1 species hybrids is caused by the segregation of incompatible genetic combinations in the F_1 microspores.

The combination of different genetic factors in the F_1 hybrids may also decrease the chiasma frequencies. Even when the genoms are similar in both morphological and genetical constitution, a genetic factor may inhibit chromosome pairing at meiosis as it does in certain cases in *Drosophila, Zea*, and *Triticum*. The degree of chromosome pairing may be greater in a generic cross than in a species cross, as PETO (13) has found in *Lolium* and *Festuca*. Such differences are evidently caused by combinations of genetic factors which influence meiosis, rather than by the degree of genetical and structural differentiation in the parental genoms. If these genetic factors cause asynapsis by delaying pairing long enough to inhibit chiasma formation, then one might expect that certain combinations of genetic factors would increase chiasma frequency by accelerating pairing. Such an effect in *Tradescantia* would increase the frequency of interstitial chiasmata. Possibly the high chiasma frequency found in the cross *T. hirsutiflora*×*T. canaliculata* is caused by genetic combinations which accelerate pairing of homologous chromosomes.

NATURAL HYBRIDS

During the course of the investigation, numerous cases of natural hybridization were found between various species in the group related to *T. virginiana*. The interested reader is referred to the taxonomic monograph by ANDERSON and WOODSON (3), where all the suspected cases are tabulated. Interspecific hybridization is apparently frequent in this group. During the last ten years the senior writer has been engaged in a study of the species problem, notably in the genera *Iris, Aquilegia, Tradescantia,* and *Uvularia*. In his experience interspecific hybridization is more general in *T. virginiana* and its near relatives than in any of the other genera studied intensively. It is planned, therefore, to make a detailed investigation of natural hybrids in *Tradescantia*, and the following is scarcely even a preliminary report on the subject. The senior writer will be glad to hear of any cases of suspected hybridization in *Tradescantia* or to assist in any way with the investigation of such cases. The results of interspecific hybridization, even between the same parental species, may be quite different, depending among other things upon the comparative frequency of the two species, the presence of polyploid races within either species (or both), and the length of time during which hybridization has been occurring. The following case of hybridization at Oakhill, Austin, Texas, is presented not so much because it is generally typical as because it has been studied more intensively than have any of the other cases which have been investigated to date. The hybrids at Oakhill occurred in the edge of a small copse of *Ilex*, scrub oaks, etc. (fig. 25). The more or less abandoned fields surrounding the wood were dry and stony, with numerous cacti. In the field along the neighboring railroad right of way, *T. humilis* was abundant. Well within the wood, *T. canaliculata* (= *T. reflexa*) (diploid race) was fairly common. Along the margin of the wood, particularly in one or two semi-shaded spots near the edge, were a number of intermediate plants. Some of them were clearly intermediate, others resembled one of the species much more than the other. Samples of all three types were collected and forwarded to the greenhouses of the Bussey Institution where they were subjected to cytological and morphological analysis over a period of two years (3).

The evidence may be summarized by saying that the plants thought to be *T. canaliculata* and *T. humilis* were cytologically regular and agreed morphologically with other representatives of these two species collected from nearby colonies where hybridization was not taking place. The suspected hybrids presented a number of cytological
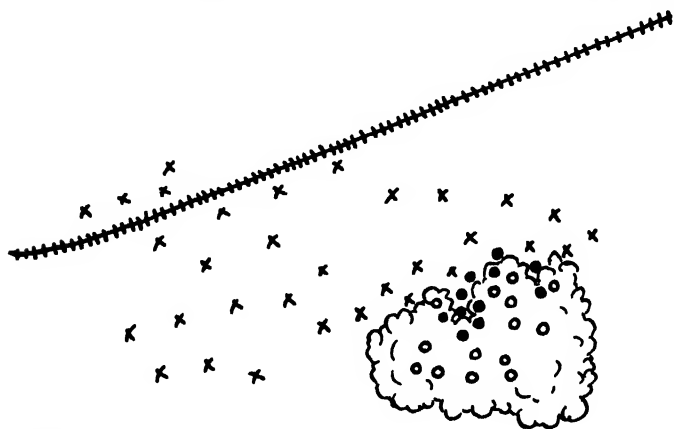
25
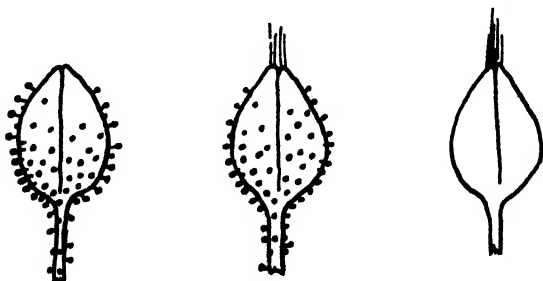
26

FIGS. 25, 26.—Fig. 25, relationships of *T. humilis* (crosses), *T. canaliculata* (open circles), and intermediates (solid black) at Oakhill, Texas. Fig. 26, sepal pubescence (somewhat diagrammatic) in, left, *T. occidentalis;* right, *T. canaliculata;* center, *T. occidentalis* within range of *T. canaliculata.*

abnormalities often associated with hybridization, namely, high percentages of sterile pollen, micro-nuclei in the tetrads, etc. Certain of the hybrids were furthermore morphologically identical with the artificial hybrids raised between the same two species, and the whole case may be taken as proved.

Since the American species of *Tradescantia* maintain themselves as recognizable units, in spite of fairly frequent hybridization, it is obvious that there are barriers which prevent more frequent admixture. A few of these can be enumerated. In the first place, as might have been suggested by the cytological evidence alone, there seems to be a complete, or practically complete, barrier to crossing between the three groups of American species, that is between (1) *T. micrantha*, (2) *T. virginiana* and its relatives, and (3) *T. rosea*. Within the Virginiana group, chromosome number is apparently important. *T. bracteata*, a diploid, is associated mainly with tetraploid races or species and hybridization is rare. *T. hirsuticaulis*, a diploid, occurs rather commonly in association with the tetraploid *T. subaspera* var. *montana*, but in spite of extensive search Dr. HIRAM SHOWALTER found very few suspected hybrids (personal communication). Such hybrids would ordinarily be triploids and practically sterile. Another barrier between *T. hirsuticaulis* and *T. subaspera* var. *montana* is the time of blooming. The former species blooms very early, and, like most of the diploids, for only a short time. For this reason crossing with the later blooming species such as *T. virginiana*, *T. canaliculata*, and *T. subaspera* var. *montana*, with all of which it is occasionally associated, is kept at a minimum.

One of the most potent barriers is the difference in habitat preference. In the Oakhill example it was the distinctive preferences of *T. humilis* and *T. canaliculata* which confined successful hybridization to a narrow zone between the sunny field and the shady wood. Farther north in the middle west *T. canaliculata* grows characteristically in a hot, dry place, often on limestone outcrops. *T. subaspera* var. *typica* is general throughout the same region but for the most part is confined to acid or subacid soils in shade or semi-shade. Over a very wide area the two species are found within a comparatively short distance of each other but are seldom found growing together because of this pronounced difference in habitat preference. In the rare instances when they do occur near together hybrids can almost always be found.

The consequences of hybridization will vary greatly with the relative abundance of the two species, with the relative size of the zone of contact, and with the presence or absence of polyploid races within

either species or both. The last point has been discussed in detail in a recent publication (3) and need not be repeated here. If the zone of contact is relatively narrow and the two species are approximately equally abundant within this zone, we shall have the simplest possible relation between two hybridizing species; two well marked species connected by a comparatively few obvious, semi-sterile intermediates. If the zone is wider the percentage of intermediates may become so great that it is no longer possible to separate the two original entities, and they will probably most readily be classified as varieties of a single species. If one of the two species is much more abundant than the other in the zone of contact, the majority of the crosses which take place will be between previous crosses (or their descendants) and the commonest parent. Such individuals, three-fourths or more of their parentage having come from the predominant parent, will not ordinarily appear as hybrids but as somewhat extreme or unusual examples of the predominant species. An interesting example of this phenomenon is apparently to be found in the relation between *T. occidentalis*, the common spiderwort of the great plains, and *T. canaliculata* (*T. reflexa*), the common species of the middle west. Both species have glaucous foliage; *T. canaliculata* is glabrous except for a tuft of eglandular hairs at the tips of the sepals (fig. 26). Outside their zone of contact *T. occidentalis* has no terminal tuft, its pubescence is glandular (fig. 26) and tends to be strongest at the base of the sepal rather than at the tip. In their zone of contact, however, *T. occidentalis* is characteristically provided with at least a few terminal eglandular hairs, a feature obviously derived from *T. canaliculata*. We apparently have here an example of a phenomenon which, for the want of a better name, may be termed "mass infection." Hybridization has continued so long and so extensively that throughout the zone of contact the germplasm of the predominant species is "infected" with a small percentage of the germplasm of the less abundant species.

Cytological survey of American Tradescantias

TECHNIQUE

To those whose acquaintance with *Tradescantia* is limited to the study of slides in the cytological laboratory, the assembling and

labeling of a collection of native Tradescantias might seem too simple a matter for scientific discussion. Experience has proved, however, that such is unfortunately not the case, and since the method finally adopted is comparatively simple and reasonably accurate, it may be briefly described. Tradescantias arrived at all times of the year and from all sorts of correspondents; they belonged to nearly thirty different species, several of these previously undescribed. For purposes of identification each collection was given a name, usually the name of the donor or of the immediate locality from which they came, and each individual plant was given a separate number. "Swarthmore 1" to "Swarthmore 13," for instance, represent thirteen plants of *T. virginiana* collected by Dr. Thomas Kerr along Crum Creek near Swarthmore College, Swarthmore, Pennsylvania. It is perhaps worth mentioning that upon arrival plants were carefully examined so that only a single individual was given a single number. With most of the northern species this was a comparatively simple matter, but with some of the southern species, notably *T. paludosa*, it was necessary to wash all the soil from the roots and make a careful examination before the plants were potted.

PRESENTATION OF DATA

The living collections, labeled and numbered as described, are summarized in table VI, where they are arranged alphabetically according to collection name. For each collection there is given the collector's name, the approximate location, the specific identification according to ANDERSON and WOODSON's monograph, and the chromosome number. To simplify the table, for those plants collected by R. E. WOODSON the collector is designated as "W." and for those collected by EDGAR ANDERSON as "A." To these various collectors we are profoundly grateful, most particularly to DR. HIRAM SHOWALTER and to D. GEORGE DIEHL, who made substantial contributions of pertinent data as well as providing the actual plants. Herbarium specimens of the collections have been made wherever possible. The first set is to be deposited at the Herbarium of the Missouri Botanical Garden, and duplicate sets, so far as they are available, will be sent to other representative taxonomic institutions.

TABLE VI

NAME	NOS.	SPECIES	CHROMOSOME NO.	LOCALITY	COLLECTOR
Alabama	1-3	T. sp.	6	Warrior River, Alabama	Miss Barbara Andrew
Algonquin	1	T. canaliculata	12	Algonquin (St. Louis) Missouri	A.
Algo flex.	1	T. canaliculata	12	" " "	W.
Algo hybrid	1-3	T. canaliculata×T. virginiana	12	" " "	W.
Algovirg.	1	T. virginiana	12	" " "	W.
Amana	1	T. canaliculata	12	Amana, Iowa	D. George Diehl
Antonio	1-2	T. canaliculata	12	Antonio, Missouri	A.
Air Mail	1	T. sp. (?)	6	Texas	B. C. Tharp
Ashland	1-6	T. subaspera typica	12	Ashland, Missouri	A.
Anniston	1	T. subaspera montana	12	Anniston, Alabama	Hiram Showalter
August	......	T. hirsutiflora	12	....	W.
Austin	1-13, 7a	T. occidentalis	6	Austin, Texas	A.
Balsam Gap	1-16	T. subaspera montana	12	Balsam Gap, North Carolina	A.
Beebe	1-9	T. occidentalis×T. canaliculata	12	Beebe, Arkansas	W.
Belew	1-3	T. occidentalis	6	Harmon, Oklahoma	Mrs. N. C. Belew
Bolton	1-4	T. canaliculata	12	Bolton, North Carolina	A.
Borglum	1-2	T. canaliculata	12	Stone Mt., Georgia	Hiram Showalter
Buda	1-0	T. gigantea	6	Buda, Texas	J. A. Devney
Bull Creek	1-3	T. edwardsiana	6	Bull Creek, Texas	A.
Cliff Springs	1-18	T. gigantea	6	Near Austin, Texas	J. A. Devney
Columbia	1-8	T. roseolens	12	Near Columbia, So. Carolina	A.
Clarke	1	T. bracteata	6	Grinnell, Iowa	Miss Cornelia Clarke
Caddo	1	T. sp.	6	Caddo Parish, Louisiana	C. Dorman
Dean	......	T. virginiana	6	Indiana	C. C. Dean
De Soto	1-6	T. virginiana	12	De Soto, Missouri	W.
Dexter	1	T. canaliculata	6	Dexter, Michigan	U. of Michigan Botanical Garden

TABLE VI—*Continued*

NAME	NOS.	SPECIES	CHROMOSOME NO.	LOCALITY	COLLECTOR
Diamond Lake	1-2	T. canaliculata	6	Diamond Lake, Noble, Co., Ind.	C. C. Deam
Edwardsiana	1-20, 15a, 10a	T. edwardsiana	6	Edwards Plateau, near Austin, Texas	A.
Elk River	1-6	T. ernestiana	6	Noel, Missouri	W.
Ellsmere	1	T. occidentalis	12	Ellsmere, Colorado	H. Stoddard
Florissant	1-2	T. canaliculata	12	Florissant, Missouri	A.
French Broad Creek	1-6	T. canaliculata×T. subaspera montana	12	Near Knoxville, Tennessee	A.
Fort Valley	1	T. virginiana	12	Fort Valley, Virginia	Hiram Showalter
Gates	1-2	T. bracteata	6	Manhattan, Kansas	David Gates
Gentilly	1-13	T. paludosa	6	Gentilly, Louisiana	A. & W.
Gravel Pit	1-4	T. subaspera montana	12	Jacksonville, Alabama	Hiram Showalter
Grinnell	20A & 20B	T. bracteata	9	Grinnell, Iowa	D. George Diehl
Hamburg	61-64, 80-84	T. canaliculata	12	Hamburg, Missouri	A.
Hair	1-5	T. subaspera montana	12	Near Jacksonville, Alabama	Hiram Showalter
Hazelton	1	T. subaspera typica	12	Hazelton, Indiana	U. of Michigan Botanical Garden
Hermann	1, P	T. subaspera typica	12	Hermann, Missouri	A.
Hillsboro	1-8	T. virginiana×T. canaliculata	12	Hillsboro, Missouri	A.
Hoffmann	1	T. virginiana	12	Sigourney, Iowa	Gerald Hoffmann
Ilex Grove	20-31	T. occidentalis	12	College Station, Texas	A.
Jacksonville	1-4	T. hirsuticaulis	6	Jacksonville, Alabama	Hiram Showalter
Jasper	1-4	T. ernestiana	6	Jasper Co., Missouri	W.
Jeanette	1-6	T. occidentalis	12	College Station, Texas	A.
Jolivette	1-5	T. canaliculata	12	La Crosse, Wisconsin	Miss Eva L. Jolivette
Kellogg	23	T. bracteata	6	Kellogg, Iowa	D. George Diehl
Killarney	1-5	T. longipes	12	Iron Co., Missouri	W.

TABLE VI—*Continued*

NAME	NOS	SPECIES	CHROMOSOME NO	LOCALITY	COLLECTOR
Knoxville	1–6	T subaspera montana	12	Knoxville, Tennessee	H M Jennison
La Barque Creek	1	T canaliculata	12	La Barque Creek, Missouri	A.
Lake Cliff		T gigantea	6	Lake Austin, Texas	J A Devney
Louisville	1–5	T. subaspera typica	12	Louisville, Kentucky	D George Diehl
Madison	1	T canaliculata	12	Arena, Wisconsin	N C Fassett
Mangelsdorf	1–21	T occidentalis	12	College Station, Texas	P C Mangelsdorf
Manhattan	1	T. bracteata	6	Manhattan, Kansas	U of Michigan Botanical Garden A.
Marthasville	21–23	T subaspera typica	12	Marthasville, Missouri	Herbert Stoddard W.
Maniton	1	T occidentalis	12	Maniton, Colorado	
Mattese	1	T virginiana	12	Mattese, Missouri	Delzie Demaree
Montgomery	1–6	T hirsuticaulis	6	Montgomery Co , Arkansas	Hiram Showalter
Mountain Lake	1	T canaliculata	12	Mountain Lake, Giles Co , Virginia	
Mt Bonnell	1–10	T gigantea	6	Mt Bonnell, Texas	J. A Devney
Mt. Mitchell	1–8	T subaspera montana	12	Mt Mitchell, North Carolina	A.
Moore's Station	6	T sp	6	Moore's Station, Iowa	D George Diehl
McKee	1–11	T canaliculata	12	State College, Mississippi	J C McKee
Mo Pac	1–15, 4W, 4V, 4T	T gigantea	6	Austin, Texas	A.
New Harmony	1	T subaspera typica	12	New Harmony, Indiana	U of Michigan Botanical Garden
N.D. & D.	1–25	T humilis	6	Austin, Texas	A
North Slope	1–6	T hirsuticaulis	6	Stone Mt , Georgia	A.
Oakhill	1–29	T canaliculata×T humilis	6	Oakhill Road, Austin, Texas	A.
Onion Creek	1–7	T gigantea	6	Onion Creek, Austin, Texas	J A Devney
Orlando	1	T canaliculata	12	Orlando, Florida	Woolman
Palmer	1	T occidentalis	12	North Dakota	Palmer

TABLE VI—*Continued*

NAME	NOS	SPECIES	CHROMO-SOME NO	LOCALITY	COLLECTOR
Pana	1	T. canaliculata	12	Pana, Illinois	A.
Perry	1-2	T. hirsutiflora	12	Athens, Georgia	Miss Lily Perry
Pierson	1	T. bracteata	6	Pierson, Iowa	D. George Diehl
Portage des Sioux	31-47	T. bracteata	6	Portage des Sioux, Missouri	A.
Portland	1	T. virginiana		Portland, Indiana	C. C. Deam
Quad petal	1	T. hirsuticaulis	6	White's Gap, Alabama	Hiram Showalter
Red Indiana		T. virginiana		Indiana	C. C. Deam
Roaring River	1	T. ozarkana	12	Roaring River State Park, Missouri	W.
Red Rock	1	T. canaliculata	12	Red Rock, Hughes Mt., Missouri	J. A. Steyermark
Reflexa	8	T. canaliculata	6	Austin, Texas	B. C. Tharp
River Cliff	1-10	T. gigantea	6	Austin, Texas	J. A. Devney
River Terrace	1-8	T. gigantea	6	Austin, Texas	J. A. Devney
Rocky Prairie	1-2	T. tharpii	12	Webb City, Missouri	W.
Royal	1	T. bracteata	6	Royal, Nebraska	U. of Michigan Botanical Garden
Royal Gorge	1	T. canaliculata	12	Royal Gorge, Missouri	J. A. Steyermark
Reita	1	T. canaliculata	12	Schoolcraft, Michigan	A. C. Anderson
San Antonio	1-5	T. sp (?)	6	San Antonio, Texas	Mrs. S. D. McKelvey
Sand Mountain	1	T. canaliculata×T hirsuticaulis	12	Muscle Shoals, Alabama	Hiram Showalter
Schoolcraft	1-3	T. canaliculata	12	Portage, Michigan	A.
Shoal Creek	1-14	T. gigantea	6	Austin, Texas	J. A. Devney
Signal	1-2	T. occidentalis	12	College Station, Texas	A.
Smith's Mills	1	T. subaspera typica	12	Smith's Mills, Kentucky	U. of Michigan Botanical Garden
Somena	1	T. occidentalis	12	Somena, Kansas	Herbert Stoddard
Starved Rock	1-3	T. canaliculata	12	Starved Rock, Illinois	Edward King
Steere	1-6	T. canaliculata	6	Ann Arbor, Michigan	W. C. Steere

TABLE VI—*Continued*

NAME	NOS.	SPECIES	CHROMOSOME NO.	LOCALITY	COLLECTOR
South Webster	1	T. canaliculata	12	Webster Groves, Missouri	A.
Swarthmore	1–13	T. virginiana	12	Swarthmore, Pennsylvania	Thomas Kerr
Showal	1	T. hirsuticaulis	6	Jacksonville, Alabama	Hiram Showalter
Sylva	1	T. subaspera montana	12	Sylva, North Carolina	Hiram Showalter
Tama	11–13	T. bracteata	6	Tama, Iowa	D. George Diehl
Tarbox	1	T. rosea	12	Brookgreen, So. Carolina	F. G. Tarbox, Jr.
Terrace	1–12	T. gigantea	6	Austin, Texas	J. A. Devney
Three Rivers	30	T. canaliculata	12	Three Rivers, Michigan	A.
Turkey Run	1–5	T. subaspera typica	12	Turkey Run, Indiana	Russell Artist
Texana	5, 5L, 5M	T. sp.	6	Austin, Texas	B. C. Tharp
Tocacco Landing	1	T. virginiana	12	Harrison Co., Indiana	C. C. Dean
Ullin	20	T. canaliculata	12	Ullin, Illinois	A.
Waterloo	1	T. canaliculata	6	Waterloo, Iowa	D. George Diehl
Warrenton	1–5	T. canaliculata	12	Warrenton, Georgia	A.
Washington University	31–34	T. canaliculata	12	St. Louis, Missouri	D. George Diehl
White's Gap	1	T. hirsuticaulis	6	White's Gap, Alabama	Hiram Showalter
Winter Haven	1–56	T. sp. (?)	6	Winter Haven, Texas	E. Mortensen
Winslow	1	T. occidentalis	12	Winslow, Arizona	Mrs. S. D. McKelvey

SUMMARY OF DATA ON CHROMOSOME NUMBERS

Broadly speaking, the American species of *Tradescantia* allied to *T. virginiana* can be divided into (1) diploid races and species, mostly southern and mostly of comparatively narrow range (fig. 27); and (2) autotetraploid species and races, mostly northern, several of them being of very wide distribution (fig. 28).

The following species are so far known only as diploids:

T. edwardsiana Tharp.—A species of very restricted distribution found in the Edwards Plateau in Texas (23). Morphological and geological evidence indicates it as probably one of the "oldest" elements among the American Tradescantias.

T. ernestiana Anderson and Woodson.—A species closely allied to the tetraploid *T. virginiana*, but restricted to a small area in the western Ozarks.

T. gigantea Rose.—A distinct and well differentiated species, restricted to a small area in central Texas but a common plant within that area. It is, incidentally, one of the best diploid Tradescantias for cytological and genetical experimentation.

T. paludosa Anderson and Woodson.—A species which roots at the nodes like many of the tropical Tradescantias. Restricted to the lower Mississippi delta. It is characterized by a high frequency of small fragment chromosomes in addition to the regular diploid complement.

T. bracteata Small.—This species has the widest distribution of any of the diploid species. It is possible that it may be a tetraploid on the northwestern border of its range since no material has been cytologically examined from that region. Like all the diploids it has a short blooming period and, since it afterwards dies down completely, it is well adapted to the hot dry summers of the western prairies.

T. humilis Rose.—A common species of eastern Texas where it is something of a weed. It apparently hybridizes more frequently with other species than do any of the other diploid species.

T. subacaulis Bush.—Restricted to a small area in east Texas.

T. hirsuticaulis Small.—A delicate montane species restricted to a narrow belt along the southern border of the Appalachians and to an isolated area on the western side of the Mississippi embayment in the

FIG. 27

FIG. 28

FIGS. 27, 28.—Fig. 27 (above), approximate distribution of diploid species of *Trades-cantia:* solid line, *T. bracteata* (middle west), *T. humilis* (Texas), *T. gigantea* (central Texas), *T. hirsuticaulis* (southern states). Stippled area, *T paludosa* (Mississippi delta), *T. subacaulis* (Texas). Solid black area, *T. ernestiana* (Ozarks), *T. edwardsiana* (Texas). Fig. 28 (below), approximate ranges of tetraploid species of *Tradescantia:* Solid line, *T. occidentalis;* coarse dashes, *T. canaliculata;* large dots, *T. virginiana* in north and *T. hirsutiflora* on gulf coast; dash and dot, *T. subaspera;* stippled area, *T. tharpii* in middle west and *T. roseolens* in Florida; solid black area, *T. longipes*.

southern Ozarks. This is a range similar to many of the typical species of this region and indicates that they have been in the region since the close of the Cretaceous (22).

The following species are known only as tetraploids, or are predominantly so:

T. subaspera Ker-Gawl.—A widespread woodland and mountain species in the Appalachians and eastern Ozarks. Its probable relations with *T. canaliculata* are discussed in detail in the section on hybridization. Known only as tetraploid (fig. 29).

T. ozarkana Anderson and Woodson.—Restricted to a small area in the western Ozarks. Known only as a tetraploid.

T. virginiana L.—Few of the plants in cultivation under this name represent the genuine species (see below). It is a widespread woodland species in the central states. Known only as a tetraploid (fig. 30).

T. hirsutiflora Bush.—Found along the coastal plain of the Gulf of Mexico from Texas to Florida. It is a ubiquitous species, apparently hybridizing freely with other species within that area. A diploid in Texas and a tetraploid in the gulf states.

T. tharpii Anderson and Woodson.—A species of the southern great plains from Texas to Kansas. Known only as a tetraploid, although probably diploid in Texas.

T. canaliculata Raf.—A ubiquitous species, a common roadside weed in the middle west, actively spreading along railroad rights of way, etc. along the boundaries of its present range. A diploid in southern Texas, tetraploid in the north, although diploid individuals have been found at other points (fig. 31).

T. longipes Anderson and Woodson.—An "ancient" species with a very restricted distribution in the Ozarks. Closely allied to the more widespread and vigorous *T. tharpii*. Apparently always a tetraploid.

T. occidentalis (Britton) Smyth.—A widespread species of the great plains and eastern Rocky Mountains. A diploid in southern Texas, tetraploid elsewhere (fig. 32).

T. roseolens Small.—Closely allied to *T. occidentalis*, but native to Florida and the adjacent coastal plains. Known only as a tetraploid.

The approximate range of each species has been determined from the accurate distribution maps published in ANDERSON and WOOD-

FIG 29

FIG 30

FIGS 29, 30 —Fig 29 (above), approximate range (dotted line) of *T. subaspera* and localities from which plants were obtained for cytological examination Fig 30 (below) approximate range of *T virginiana* and location of wild growing plants obtained for cytological examination.

FIG 31

FIG 32

FIGS 31, 32 —Fig 31 (above), approximate range (dotted line) of *T canaliculata* and localities from which plants were obtained for cytological examination Solid black, tetraploid, open circles, diploid Fig 32 (below), approximate range of *T occidentalis* and localities from which plants were obtained for cytological examination Solid black, tetraploid; open circles, diploid

SON's monograph (3) and is summarized in table VII. The average range in square miles for the diploid species is 83,475; for the tetraploid species, 376,300. Since the spreading power of a species is more accurately reflected by the radius of its distribution area rather than by the area itself, we have extracted the square roots of these two figures, obtaining an average "diameter" for the tetraploid species of

TABLE VII

TRADESCANTIA SPECIES ALLIED TO T. VIRGINIANA,
WITH THEIR APPROXIMATE RANGES IN
SQUARE MILES

	RANGE IN SQ. MILES
Species known only as diploids:	
T. edwardsiana	5,300
T. ernestiana ...	26,500
T. gigantea...	10,600
T. paludosa..	47,700
T. bracteata .	381,600
T. hirsuticaulis..	37,100
T. humilis.....	143,100
T. subacaulis..	159,000
Species known only as autotetraploids, or predominantly so:	
T. subaspera...................	371,000
T. ozarkana...	10,600
T. virginiana..	397,500
T. hirsutiflora..	265,000
T. tharpii.......	132,500
T. canaliculata....	1,166,000
T. longipes........	10,600
T. roseolens........	79,500
T. occidentalis........	1,166,000
Average range of diploids... .	83,475 sq. miles
Average range of tetraploids..	376,300 sq. miles

613 miles and for the diploids of 289 miles. The ratio between these two diameters is 2.1 to 1; that is, a tetraploid species on the average has just about twice the radius of a diploid species.

Reports of intraspecific autopolyploidy in *wild* species, while not unknown (4, 11), are somewhat rare and for the most part are from genera, such as *Nasturtium*, in which vegetative propagation is well developed. A whole group of vigorous autopolyploid species, such as these Tradescantias, actively reproduced by seed (1) is therefore an

exceptional case. This section of the genus *Tradescantia* should there-
fore possess certain basic characteristics which make possible such an
extensive and successful development of autopolyploidy. Two such
characteristics may be suggested, terminal chiasmata and median
attachment constrictions.

With completely terminalized chiasmata the configurations are
fairly complicated, and occasionally lead to numerical non-disjunc-
tion, chromosome interchange, and other irregularities, but they are
simple in comparison with what would have resulted with a high
chiasma frequency and little or no terminalization. Under these lat-
ter conditions the configurations are obviously too complicated to
permit regular disjunction. In this connection the range of *T. gi-
gantea* is of interest. It is confined to an exceedingly small area in
central Texas, yet within that area it exhibits many of the charac-
teristics of a potential weed. It is, however, uniformly diploid. Ex-
tensive samplings of wild populations (93 plants from nine localities)
have revealed only diploid plants. This is noteworthy in view of the
fact that *T. gigantea* has a consistently higher chiasma frequency
than *T. canaliculata* and *T. occidentalis* (table II). It seems not im-
probable that the absence of polyploid strains within *T. gigantea*
may be due, not to their never having arisen, but that once orig-
inated their reduction divisions were too complicated to permit
natural survival. A second characteristic of these Tradescantias
permitting regular disjunction in autotetraploids is median or sub-
median attachment constrictions.

CONSEQUENCES OF AUTOPOLYPLOIDY

It might confidently be predicted that autopolyploidy would pro-
duce individuals differing somewhat from the diploids from which
they arose. Since from the simplest *a priori* assumptions some of the
reactions of the nucleus are functions of its volume and some are
functions of its surface, doubling the chromosome number (and
hence doubling the volume, with a consequent increase in the surface
of barely 60 per cent) should alter the general metabolic rate of the
cell. As a matter of fact most autotetraploids differ from the diploids
from which they arose, some of them markedly. In *Tradescantia* the
difference is slight but perceptible. Two species, *T. canaliculata* and

T. occidentalis, have extensive diploid and tetraploid races which have been investigated during the course of this investigation. We have never been able to classify plants of either species as diploids or tetraploids by their appearance alone. Once classified by cytological examination, however, it has always been easy to see that the tetraploids are on the whole definitely larger and that they have much longer blooming seasons. This has been equally true of plants cultivated in the breeding plots during the summer and of those grown in pots in the greenhouse during the winter. As a consequence of their greater vigor the tetraploid species and races have a much greater colonizing ability, as has already been demonstrated. The cases of *T. canaliculata* and *T. occidentalis* are particularly interesting, each apparently having originated in the south as a diploid while autopolyploid races subsequently arose in each species and spread extensively over more than a million square miles, *T. occidentalis* on the great plains and *T. canaliculata* on the prairies and along the coastal plain. Each is a common weed in its own territory and is actively enlarging its boundaries at the present time. Most of the other tetraploid species present a similar picture, notably *T. hirsutiflora* which, a diploid in central Texas, has spread north and east as a tetraploid weed.

This entire section of the genus *Tradescantia* presents therefore an interesting example of parallel evolution. Endowed through their common origin with the same basic abilities, many of the species have evolved in a similar fashion. With genoms characterized, among other things, by median attachment constrictions and terminal chiasmata, several of the species have been able, independently, to produce vigorous autotetraploid races which were prepared to travel even faster and farther when white civilization appeared in North America.

Comparison of cultivated and indigenous Tradescantias

With many cytologists and geneticists the opinion has been prevalent that there is no real distinction between cultivated plants and wild species, or at least between cultivated plants run wild and the indigenous elements of a flora. While the question has seldom been argued in print, it has (outside the Scandinavian countries, at least)

been tacitly taken for granted as a working hypothesis by many if not most cytogeneticists. Certainly the senior writer has argued the question frequently and fruitlessly with many of his cytogenetic colleagues. It is gratifying, therefore, to be able to present not only the taxonomic evidence and the *a priori* reasoning which led him originally to his own point of view, but also a body of cytological facts, statistically summarized and pointing to the same conclusion.

The taxonomic evidence may briefly be summarized by saying that the majority of taxonomists consider cultivated plants as belonging to a somewhat different category from the strictly indigenous elements of a flora. Reasoning *a priori* from the known facts of cytology and genetics one might reach similar conclusions. Certainly the percentage of recessives, the opportunities for interspecific hybridization, for chromosome interchange, and for the survival of most cytological irregularities would be greatly different among cultivated plants from what obtained in the areas from which these plants were ultimately derived.

Fortunately in *Tradescantia* we have a remarkably complete test case. The common garden spiderwort is known to have been derived *in toto* from the American species closely related to *T. virginiana*, that species and *T. subaspera* Ker-Gawl. (= *T. pilosa* Lehm.) having been the most important contributing species. In the more than 300 years since their introduction to cultivation they have had the opportunity in European gardens, away from their original home, to run the usual course of a cultivated ornamental; to suffer periods of fashion and periods of neglect; to be consciously and unconsciously hybridized and selected, and to run wild again at certain points. In 1930 a representative sample of these cultivated spiderworts was brought together and analyzed cytologically (6). With the resources of the John Innes Horticultural Institution, the collection was a representative one and the cytological work is likewise unimpeachable. It forms an unusually trustworthy body of evidence, therefore, with which to compare the data on wild spiderworts compiled in the course of this investigation. The comparison (table VIII) is made by percentage frequencies for the various cytological types in the cultivated English plants on the one hand (*loc. cit.* p. 218) and for some 268 wild American plants for which definite records had been kept. Table

VIII shows how the cultivated plants give an erroneous impression of the percentages of triploids and of extra-chromosomal types, and fail to reveal the important diploid species. The interested reader is referred to the original paper (6) from which the data on the cultivated European plants were taken. It will be seen that although the investigation was avowedly (*loc. cit.* p. 207) an attempt to apply cytological methods to taxonomic questions, the failure to discriminate between the cultivated "*T. virginiana* L. U.S.A." and its wild progenitors led to erroneous conclusions as to the importance of vege-

TABLE VIII

COMPOSITION OF WILD AND CULTIVATED TRADESCANTIAS.
PERCENTAGE COMPOSITION OF T. VIRGINIANA AND ITS
CLOSE RELATIVES AS INDICATED BY WILD AND BY CUL
TIVATED PLANTS

	PERCENTAGE	
	CULTIVATED	WILD
2n	0 0	55.6
3n	5.3	0 4
4n	57.9	41 0
4n+f	26 3	3 0
4n+1	5 3	0.0
4n+1+f	5 3	0.0

tative reproduction (p. 254) and the relationships of the Texas species (p. 279).

The conclusion seems inescapable that the cultivated spiderwort, the so-called *T. virginiana* of European gardens, is quite a different assemblage cytogenetically from the native American species of *Tradescantia* from which it was ultimately derived. If this is true of *Tradescantia*, cultivated only 300 years, how much more different cytogenetically from their ultimate wild progenitors must be our common domesticated plants and animals.

Summary

1. Autopolyploidy, aneuploidy, segmental interchange, fragmentation, and differences in chromosome size were found in the nine

genera and thirty-two species of the Commelinaceae which were investigated cytologically.

2. Chromosome pairing has been studied in five diploid species of *Tradescantia*. The average number of chiasmata per bivalent is found to be fairly constant for any one plant but varies widely from plant to plant, even within the same species.

3. Pollen sterility varies from 82 to 98 per cent among the diploid species and ranges from 71 to 89 per cent among the autotetraploids.

4. An extensive cytological survey of the species allied to *T. virginiana* shows them to be made up largely of (1) diploid races and species, mostly southern, and (2) autotetraploid races and species, mostly northern in distribution.

5. The autotetraploids have roughly twice the average radius of distribution of the diploids.

6. Terminal chiasmata and median attachment constrictions, conditions favoring autotetraploidy, are discussed.

7. Autotetraploid Tradescantias are slightly larger and have longer blooming periods than the related diploids, but can be distinguished from them only cytologically.

8. Cytological conditions favoring autotetraploidy have brought about the independent evolution in different species of *Tradescantia* of vigorous autotetraploid races.

9. Five artificial interspecific diploid hybrids were produced experimentally and studied cytologically. Pairing in these hybrids is regular although the chiasma frequency is slightly lower than the average of the parents. Pollen fertility varies from 40 to 73 per cent. The evidence therefore indicates that structural changes have played little or no part in the immediate differentiation of these species.

10. Natural hybrids are discussed and illustrated by examples.

11. Certain of the internal and external barriers to interspecific hybridization in the American Tradescantias are enumerated.

12. The various consequences of hybridization under different conditions are described.

13. The cultivated Tradescantias are compared with the wild species from which they were ultimately derived and their cytological differences are compared statistically.

Conclusions

EVOLUTIONARY PROCESSES IN THE COMMELINACEAE

Structural change has evidently been an important factor in the differentiation of genera. Polyploidy (allopolyploidy, autopolyploidy, aneuploidy), segmental interchange, fragmentation, and differences in chromosome size are the most conspicuous changes which have occurred.

EVOLUTIONARY PROCESSES IN T. VIRGINIANA AND ITS RELATIVES

1. A special feature of evolution in this group is autopolyploidy. Rare (or at least seldom reported) in other strictly wild species, it is here the rule. Conditions favoring its development have encouraged the independent evolution in the whole group of species, of vigorous autotetraploid races. Autotetraploidy, although it complicates the relationships between species, and although it enriches the pattern of variation within species, cannot be considered a factor of major evolutionary importance, even in these Tradescantias.

2. Factors of general evolutionary importance here are structural changes, hybridization, and genic differentiation:

(a) *Structural changes.*—Various examples were occasionally found, as for instance, fragmentation and chromosome interchange. There was, however, no evidence that these factors had been of any importance in the immediate differentiation of these species.

(b) *Hybridization.*—It is in *Tradescantia* an evolutionary feature of particular importance, deserving of careful field analysis. Its effects are various, depending upon both internal and external variables.

(c) *Genic differentiation.*—Apparently the major factor in the differentiation of these species has been the slow accumulation of genic differences. As to when, where, or how these hypothetical genic differences have accumulated we have as yet no evidence. For the present we can only dignify our ignorance with a scientific term and conclude that "genic differences" have been the most important factor in the immediate development of these American Tradescantias.

The writers have carried out the work reported here while serving as members of the staff of the Arnold Arboretum of Harvard Uni-

versity. The actual growing of the living material and its cytological examination have taken place at their alma mater, the Bussey Institution of Harvard University. For the privilege of sharing in the stimulating atmosphere and material advantages of this latter laboratory of which they are staff members only by courtesy, they are profoundly grateful.

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LITERATURE CITED

1. ANDERSON, EDGAR, and DIEHL, D. G , Contributions to the *Tradescantia* problem. Jour. Arnold Arb. 13:213–231. 1932.
2. ANDERSON, EDGAR, and SAX, KARL, A cytological analysis of self-sterility in *Tradescantia*. BOT. GAZ. 95:609–621. 1934.
3. ANDERSON, EDGAR, and WOODSON, R. E., Contrib. Arnold Arb. of Harvard University. No. 9. 1935.
4. BLACKBURN, K. B., On the relation between geographical races and polyploidy in *Silene ciliata* Genetica 15:49–66. 1933.
5. BRÜCKNER, GERHARD, Commelinaceae. In ENGLER and PRANTL, Naturliche Pflanzenfamilien. 2 ed. 15a:159–181. 1930.
6. DARLINGTON, C. D., Chromosome behavior and structural hybridity in the Tradescantiae. Jour. Gen. 21:207–286. 1929.
7. ———, Recent advances in cytology. pp. 559. Blakiston, Philadelphia. 1932.
8. HUSKINS, C. L., and SMITH, S. G., Meiotic chromosome structure in *Trillium erectum* L. Ann. Bot. 49:119–150. 1935.
9. IWATA, J , Chromosome structure in *Lilium*. Mem. Coll. Sci. Kyoto Imp. Univ. 10:275–288. 1935.
10. KING, EDWARD, Chromosome behavior in a triploid *Tradescantia*. Jour. Hered. 24:252–256. 1933.
11. MANTON, IRENE, The cytological history of watercresses (*Nasturtium officinale* R. Br.). Zeitschr. Indukt. Abstam. Vererb. 69:132–157. 1935
12. MATHER, K., Meiosis in *Lilium*. Cytologia 6:354–380. 1935.
13. PETO, F. H., The cytology of certain intergeneric hybrids between *Festuca* and *Lolium*. Jour. Gen. 28:113–156. 1934.
14. RILEY, H. P., Effect of X-rays on the chromosomes of *Tradescantia*. Cytologia (in press).
15. SAX, KARL, The cytological mechanism of crossing over. Jour. Arnold Arb. 13:180–212. 1932.

16. SAX, KARL, Chromosome ring formation in *Rhoeo discolor*. Cytologia **3**:36–53. 1931.

17. ———, Chromosome structure in the meiotic chromosomes of *Rhoeo discolor* Hance. Jour. Arnold Arb. **16**:216–224. 1935.

18. ———, Variation in chiasma frequencies in *Secale*, *Vicia* and *Tradescantia*. Cytologia **6**:289–293. 1935.

19. SAX, KARL, and ANDERSON, EDGAR, Segmental interchange in chromosomes of *Tradescantia*. Genetics **18**:53–67. 1933.

20. SAX, KARL, and EDMONDS, H. W., Development of the male gametophyte in *Tradescantia*. BOT. GAZ. **95**:156–163. 1933.

21. SAX, KARL, and HUMPHREY. L. M., Structure of meiotic chromosomes in microsporogenesis of *Tradescantia*. BOT. GAZ. **96**:353–362. 1934.

22. STEYERMARK, J. A., Some features of the flora of the Ozark region in Missouri. Rhodora **36**:214–233. 1934.

23. THARP, B. C., Rhodora **34**:57–59. 1932.

EFFECTS OF NUTRIENT CONCENTRATION ON ANATOMY, METABOLISM, AND BUD ABSCISSION OF SWEET PEA

G. T. NIGHTINGALE AND R. B. FARNHAM

(WITH TWENTY-ONE FIGURFS)

Introduction

For several years the writers have been conducting experiments concerned with the premature abscission of flower buds of sweet pea, *Pisum lathyrus odoratus*. Under commercial greenhouse conditions the sweet pea is often grown in a fertile soil high in organic matter and plentifully supplied with moisture. Under such treatment, a vigorous succulent growth usually occurs, accompanied, in the short cloudy days of winter, by abscission of a high percentage of the buds. Experimentally plants have been grown under different environmental conditions and with varied nutrient media, but throughout the course of the work one fact stood out clearly: abscission of the buds was intimately associated with the character of growth of the plants. It occurred to the greatest extent on plants that grew vigorously, were comparatively succulent, and had thin dark green leaves of relatively large area.

Chemical analyses of such plants showed that they were invariably high in percentage concentration of amino acids and proteins but low in reserve carbohydrates. Limitation of the supply of available nitrogenous nutrient seemed therefore to be indicated as a probable means by which plants having a lower concentration of organic nitrogen and a higher concentration of carbohydrates could be grown less vigorously vegetative. A low plane of nitrogen supply in sand culture tended to give this result and also a decreased percentage of bud drop. In the case of sweet peas under commercial conditions, however, when the daily and seasonal variations in sunlight and, therefore, in rate of carbohydrate synthesis were very great, it was found difficult on the one hand to avoid extreme nitrogen deficiency and carbohydrate accumulation resulting in a small yield of flowers

with short peduncles; on the other hand it was found equally difficult
to avoid excessive vegetative growth, which was associated with ab-
scission of buds and a high percentage of elaborated nitrogen in the
tissues of the plant but a low concentration of carbohydrates.

Observations of the practices of a few growers who were more suc-
cessful than the average in controlling abscission of flower buds indi-
cated that in general they employed soils which were high in concen-
tration of plant nutrients, including nitrate. During the winter peri-
od, however, when photosynthetic activity was limited by relatively
little sunlight, they watered the plants sparingly although the plants
were not permitted to wilt. Under such treatment there was pro-
duced a moderately vigorous vegetative growth that was not exces-
sively succulent, fewer flower buds abscissed, and a good yield of
flowers was obtained. The plants did not seem to be materially lim-
ited in their capacity to absorb nutrients because of the low supply
of water in the soil. Nitrate and mineral nutrients occurred in abun-
dance in the tissues of roots and tops. Nevertheless, the plants were
not high in concentration of the elaborated forms of nitrogen such as
amino acids and proteins, but did contain a relatively high percent-
age of reserve carbohydrates.

Assuming that one of the principal effects of a low soil moisture
content was an increase in the concentration of salts in the soil solu-
tion, tests in sand culture were made. High and low concentrations
of a complete nutrient solution containing abundant nitrate were
employed. The results of these experiments are discussed in the fol-
lowing pages.

Experimental methods

On August 4, 1934, seeds of the variety Balls Rose were planted
in white quartz sand in 12-liter self-draining porcelain jars. A few
days after the tops emerged above the sand the plants were selected
for uniformity and were thinned to two plants per jar. All received
the nutrient solution indicated in table I, made up with tap water at
an approximate concentration of one half atmosphere. One liter of
solution was supplied as one application per day to each culture.
After September 30, the solution was percolated through the sand
according to the constant renewal method of SHIVE and STAHL (31),
and the amount of solution was increased to 2 liters per culture per

24 hours. Also at that time the plants were divided into four groups of thirty-two plants each, each group receiving the same proportion of nutrient salts but at approximate concentrations of one half, one, two, and three atmospheres respectively. On November 1, the amount of solution percolated through the sand was increased to 3 liters per culture per day.

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS

APPROXIMATE CONCEN-TRATION OF NUTRIENT SOLUTIONS IN ATMOSPHERES	PARTIAL VOLUME MOLECULAR CON-CENTRATION OF SALTS EMPLOYED		
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
½	0 0022	0 0045	0 0022
1	0 0045	0 0090	0 0045
2	0 0090	0 0180	0 0090
3	0 0135	0 0270	0 0135

The pH estimations of nutrient solutions were made colorimetrically before and after passing through the sand. As applied, the solutions (adjusted with KOH) were approximately pH 5.6 but after passing through the sand they frequently increased in acidity to pH 4.8.[1] The effect of such increase in acidity was minimized by frequent flushing of the cultures with 0.02 normal KOH, followed immediately with fresh nutrient solution. The pH range of the cultures from 5.6 to 4.8 is such, however, as to permit good growth and assimilation of nitrate (13, 19, 32) if other environmental conditions are favorable.

Aside from the sand culture treatments, the usual commercial procedure was followed in growing the plants. In so far as seasonal conditions permitted, the air temperature of the greenhouse was maintained at approximately 45° F. at night, during the day at about 50° if the weather was cloudy, and at 60° to 65° F. on bright sunny days. The relative humidity of the air was kept at about 75 to 85 per cent, preferably at the higher figure.

[1] When the sole external source of nitrogen is from a nitrate salt, as in the case of these experiments (19), cultures of non-leguminous plants commonly increase in alkalinity rather than acidity.

Boron, manganese, and iron were apparently present in sufficient quantity in the salts employed or in the tap water. At least additions of these elements to some of the cultures of each series produced no noticeable effect.

The plants were divided for macroanalysis into (1) leaf blades, (2) stems and petioles, and (3) roots, the last fraction including also such portion of the stem as occurred beneath the surface of the sand.

Determinations of nitrogenous and carbohydrate fractions were made with fresh and dried tissue respectively according to methods recently described (4, 22). ECKERSON'S (10) microchemical technique was followed and her procedure was employed in making reducase determinations (7).

In the preparation of permanent slides, well recognized methods were followed. Plant material was fixed in Navashin's solution, dehydrated, imbedded in paraffin, sectioned and stained with safranin and gentian violet. All drawings were made on the same scale with the aid of a projectoscope.

Records of yields and quality of flowers were taken and will be summarized in the following pages, but the junior writer will report the detailed results in another publication concerned with the use of different nutrient media and the direct commercial application of the principles which are emphasized in the following discussions.

Results

EXTERNAL APPEARANCE

The obvious effects on the plants of the respective concentrations of nutrient salts became evident rather gradually following the shift in treatments on September 30. After a week or ten days there was a noticeable effect on color of foliage, which in two or three weeks was followed by distinct differences in character of growth of all organs of the plant. Comparative differences in volume of growth as they occurred on December 9 are given in table II, and photographs taken a few days later are shown in figures 1, 2, and 3.

The plants grown in nutrient solutions of concentrations of one half and one atmosphere, respectively, were very similar in volume and quality of growth. Plants in the higher concentrations of two and three atmospheres were also similar to each other (table II, figs.

1, 2). For convenience the similar groups will be described together. Those at one half and at one atmosphere are designated as low concentration cultures and the plants at two and at three atmospheres as high concentration cultures.

Roots.—Figure 1 shows a typical pair of root systems from a single culture jar of the low and from a single culture jar of the high concentration groups. This illustration together with the data of table II shows clearly that there was a much greater volume of roots produced at low than at high concentration of nutrients. Of equal

TABLE II

COMPARATIVE AVERAGE GROWTH PER PLANT OF SWEET PEA; DECEMBER 9, 1934

| | APPROXIMATE CONCENTRATION OF NUTRIENT SOLUTION IN ATMOSPHERES | | | |
	$\frac{1}{2}$	1	2	3
Total green weight per plant				
Blades (gm.)...............................	82	99	76	67
Main stem plus lateral stems plus petioles (gm.)...................................	166	170	101	99
Roots (gm.).............	66	57	40	38
Total linear growth per plant				
Main stem plus lateral stems (cm.).........	1400	1400	1000	950

significance is the character or quality of the roots produced. At the lower two concentrations the roots were glistening white and typically of larger diameter. Except in the case of the two or three main roots the cortex was obviously alive during the whole period of the experiments. The roots were not brown even at their older or proximal ends. They were highly succulent and lacking in mechanical strength.

In contrast, the roots of the high concentration cultures were typically woody, mechanically strong, and distinctly lacking in succulence except near the distal end. Early maturity of the cortex was not due to plasmolysis of the cortical cells by the concentration of nutrient salts employed. Root hairs were present in abundance and showed no indication of plasmolysis, although they persisted for only a short time because of the rapid loss of cortical tissue.

The quality of growth of sweet pea roots at high salt concentra-
tions was very similar to that exhibited by the roots of apple and
peach trees, which were grown with the same kind of nutrient solu-
tion used here for sweet pea at a concentration of one atmosphere but
at a temperature of 75° F. and higher. Likewise, the effects of a low
concentration of nutrients on sweet pea roots were essentially similar
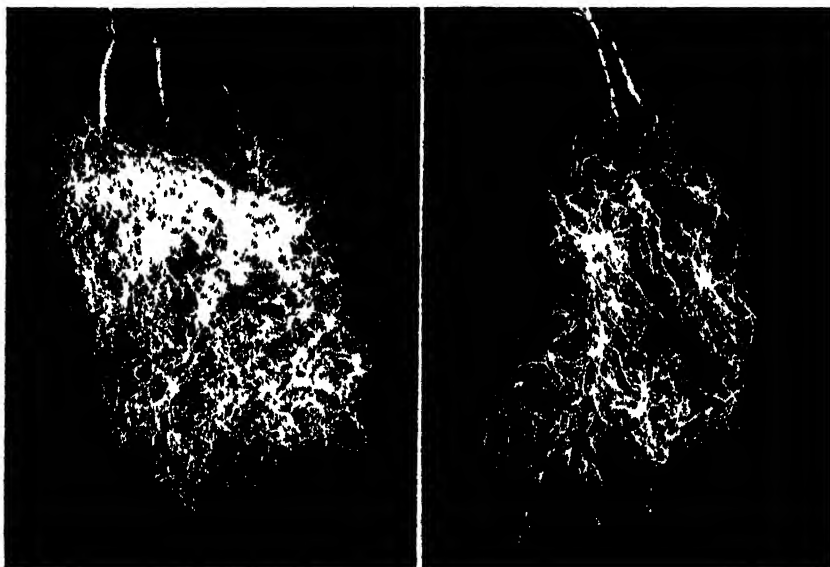
FIG. 1.—Sweet pea roots Dec. 9 Pair of root systems (left) supplied with dilute
nutrient solution (one half atmosphere). Note roots are white and that secondary
laterals are of relatively large diameter. They were succulent and matured slowly
Pair of comparable root systems (right) from single culture jar supplied with concen-
trated nutrient solution (three atmospheres). Note smaller volume of growth, that roots
are not white, and that secondary laterals are of small diameter. They were relatively
woody and matured rapidly.

to the effects on apple and peach tree roots of temperatures of 65° F.
and lower (20). In both cases the tops of the peach and apple trees
referred to were exposed to an air temperature of 65° F. In other
publications there are discussed effects of air temperature on the
growth of the tops of plants (18, 22, 23).

STEMS.—The effects of the respective nutrient treatments on the
relative amount of leaf and stem growth are indicated in table II and

FIG 2 —Tops of sweet pea plants Dec 9   Four plants (left) grown two to a culture
jar supplied with dilute nutrient solution (one half atmosphere)   These plants grew
vigorously, were dark green, comparatively soft and succulent, and many flower buds
abscissed. Comparable group of plants (right) grown in concentrated nutrient solution
(three atmospheres)   Note smaller volume of growth   These plants were lacking in
succulence, light green, and relatively few flower buds abscissed.

FIG 3 —Leaves (left) typical of plants supplied with dilute nutrient solution (one half atmosphere) Note comparatively large area and nearly circular shape Blades were thin, succulent, and dark green Leaves (right) typical of plants supplied with concentrated nutrient solution (three atmospheres) Note small area and elliptical shape They were thick, relatively less succulent, and yellowish green.

Flowers and buds (left) from plants grown in dilute nutrient solution Peduncles soft and succulent, straightening prematurely, accompanying which buds became yellowish and abscissed Buds and flowers (right) from plants grown in concentrated nutrient solution Peduncles shorter, less succulent, remaining downwardly curved until full bloom Comparatively few flower buds abscissed

figure 2. The quality of growth and yield of flowers were definitely influenced by the concentration of salts employed. The greater total linear growth of stems at the lower concentrations was due principally to the development of many lateral shoots. The stems of the plants grown at low concentrations were also much larger in diameter than at the higher concentrations, much more succulent, the internodes were longer, and the winglike lateral expansions of the stem were darker green and very large, making the stem appear relatively flat. The high concentration cultures had stems of lighter green, which were typically rather woody except near the tips, and the lateral expansions were smaller and persisted for a shorter time. The stems were accordingly more or less circular in cross section except near the distal end.

LEAVES.—The differences in amount and character of the foliage of the respective groups were also striking. The total green weight of the blades was much greater at one half or one atmosphere than at the higher concentrations (table II), but perhaps of even greater significance was the marked difference in quality of the leaves. The blades of the low concentration cultures were dark green, nearly circular in outline (fig. 3), very thin, extremely succulent, and they wilted almost immediately after picking. The petioles and tendrils were likewise succulent, relatively long, and of comparatively large diameter. The latter were frequently more or less foliate. In contrast, the blades of the plants of the high concentration cultures were typically lighter green, much thicker, almost leathery to the touch, smaller in area, and oval to eliptical in shape (fig. 3). Even an hour or more after picking and in the absence of an external supply of water, the leaves showed little evidence of wilting. Coupled with these properties the development of tendrils was much less extensive, they often exhibited a reddish tinge, and they, as well as the comparatively short petioles, were of small diameter and lacking in succulence.

These characteristics of leaves and stems are not peculiar to the effects of the concentration of salts in the nutrient medium, nor are they limited to the sweet pea. Some of the many factors (15, 18, 22, 23, 24, 27, 28) which have directly or indirectly resulted in similar responses in other plants will be discussed in the following pages. It

may be pertinent, however, to point out that in case of apple, BLAKE and DAVIDSON (1) have found that with varying degrees of vegetative vigor and fruitfulness there was variation in shape of the spur leaves, from more or less circular in case of the strongly vegetative type to oval or eliptical in case of the leaves borne on spurs that were typically less vigorously vegetative and characteristically produced fruits of high color for the variety. These differences in shape of the leaves are therefore very similar to those recorded for sweet pea, differences which in apple furnish for fruit growers an index of the degree of vegetative and reproductive vigor of their trees.

TABLE III

ABSCISSION OF BUDS AND LENGTH OF PEDUNCLES OF SWEET PEA

| ATMOSPHERES | APPROXIMATE CONCENTRATION OF NUTRIENT SOLUTION | | | | | |
| | PERCENTAGE OF BUDS THAT ABSCISSED | | | AVERAGE LENGTH (CM.) OF PEDUNCLES* | | |
	NOVEMBER	DECEMBER	JANUARY	NOVEMBER	DECEMBER	JANUARY
½................	46	59	31	26	23	23
1........................	33	47	26	26	20	23
2........................	12	30	7	25	22	16
3......	10	24	3	24	20	15

* Peduncles from buds that abscissed are not included in averages; as a rule they were 5 to 6 cm. longer than the average.

FLOWERS.—Associated with the vegetative responses already described there occurred in the low concentration cultures a much higher percentage of blossom bud abscission than in those of high concentration (table III, fig. 3). After a few days of cloudy weather the developing vegetative growth of the plants supplied with the low concentrations of nutrients always became extremely soft and there was frequently nearly 100 per cent loss of blossom buds. This was demonstrated repeatedly, and often a week or more before the flower buds actually abscissed the peduncle would straighten (fig. 3) instead of remaining in the usual downwardly curved position. Accompanying this response, the buds gradually changed from dark to yellowish green. Extremely soft, succulent growth of the vegetative organs

was invariably accompanied by the development of soft succulent peduncles and the straightening and change in color described, and by an increase in percentage of abscission.

In general the peduncles in this series were relatively long, especially in the case of those buds which abscissed. Those flowers which persisted were borne on the shorter peduncles (table III).

The less succulent plants of the high concentration cultures were correspondingly low in percentage loss of flower buds and had peduncles which remained downwardly curved, were less succulent, of smaller diameter, and shorter. Also the flowers from these non-succulent plants were much superior in keeping quality to those borne by the more succulent plants. Cultures of the latter group produced 35 per cent fewer marketable inflorescences, although when the buds persisted there were, as a rule, a larger number of individual flowers per inflorescence.

ANATOMICAL STRUCTURE

In anatomical structure, as well as in external appearance, the plants of the groups at one half and one atmospheres were very similar, as were those at two and three atmospheres. It will be unnecessary, therefore, to discuss separately the plants of the four different nutrient treatments (table I). The anatomical drawings described were made from representative plants of cultures which were supplied with the nutrient solution at a concentration of one half atmosphere and three atmospheres, respectively.

ROOTS.—It has already been recorded that the plants which received the lower concentrations of nutrient salts produced root systems that remained white and relatively succulent for the entire duration of these experiments (fig. 1), whereas at higher concentrations the cortex rapidly became brown and the central cylinder tough and woody. The associated anatomical situation is shown in figures 4–10.

It is apparent that the rate of maturity of tissues was much slower at low than at high concentrations of nutrient salts. At 1 cm. from the root tip developed in low concentration solutions there were practically no root hairs, the cortical cells were comparatively angular, closely packed, and Casparian strips were not yet noticeable
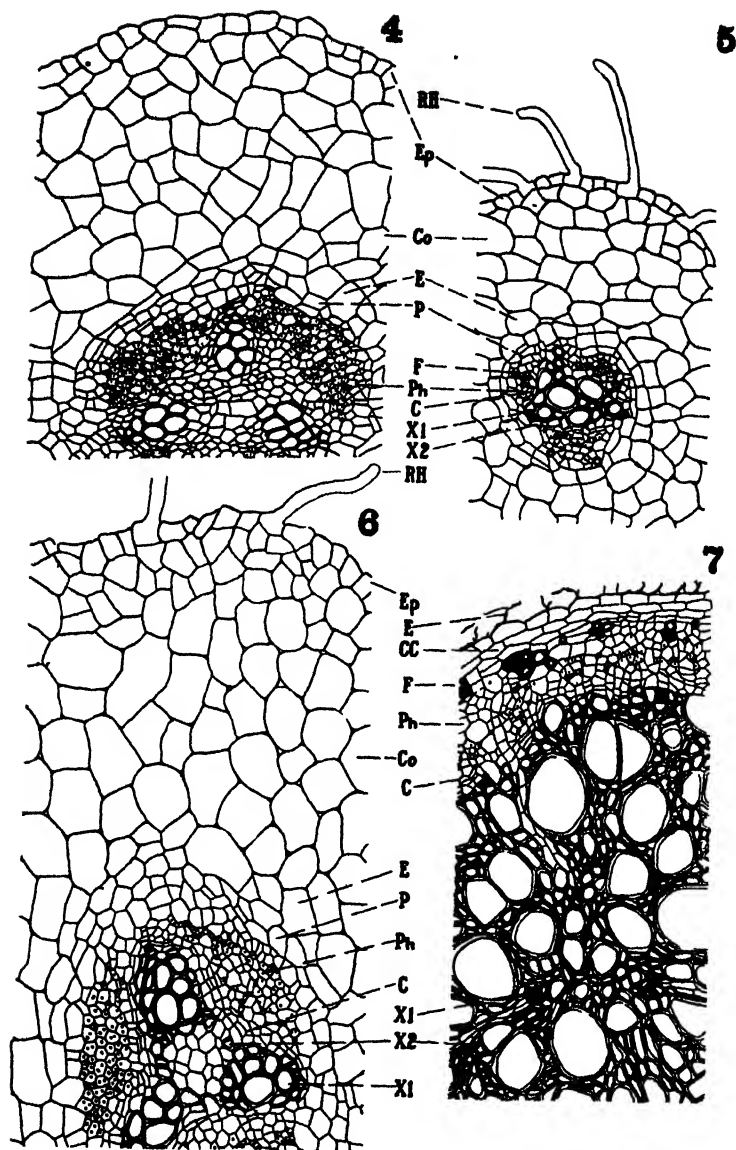
FIGS. 4–7.*—Transverse sections of sweet pea roots: fig. 4, 1 cm. from tip, dilute nutrient solution; fig. 5, 1 cm. from tip, concentrated nutrient solution; fig. 6, 5 cm. from tip, dilute nutrient solution; fig. 7, 5 cm from tip, concentrated nutrient solution. Note early differentiation and maturity of tissues (figs. 5, 7); cortex sloughed off (fig. 7).

* Abbreviations for figs. 4–21: *Ep*, epidermis; *Co*, cortex; *E*, endodermis; *CC*, cork cambium; *P*, pericycle; *F*, fibers; *Ph*, phloem; *C*, cambium, *X1*, primary xylem; *X2*, secondary xylem; *Pi*, pith; *M*, mesophyll, *S*, stoma; *UEp*, upper epidermis; *LEp*, lower epidermis; *AZ*, abscission zone.
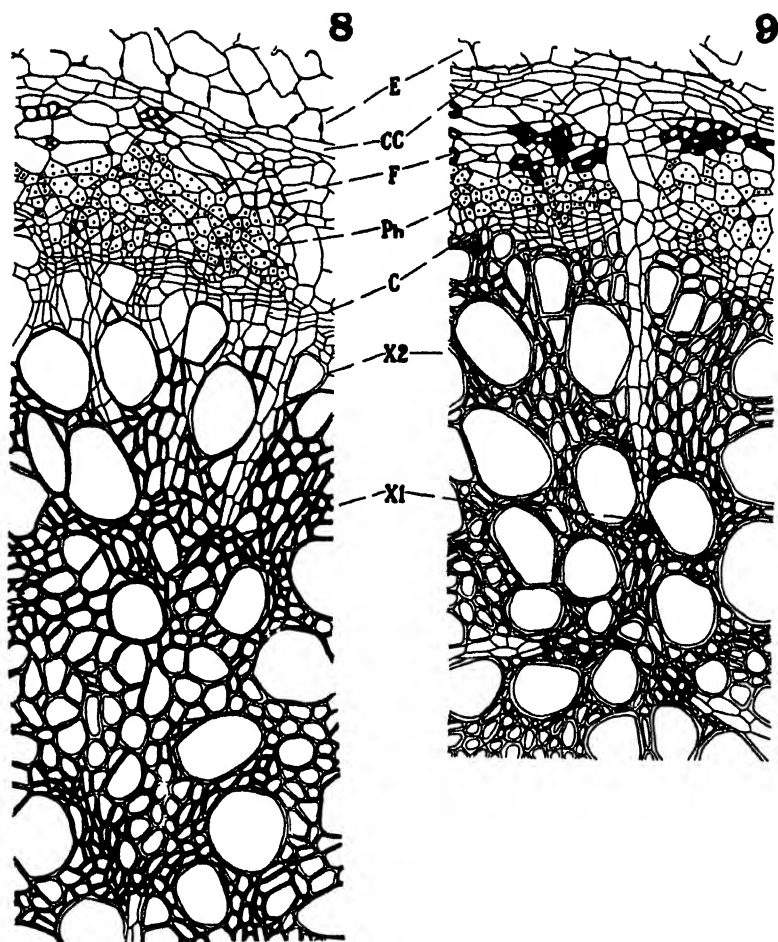
FIGS. 8, 9.—Transverse sections of sweet pea roots 15 cm. from tip: fig. 8, dilute nutrient solution (cortex alive but not shown); fig. 9, concentrated nutrient solution (cortex sloughed off heavy fibers and few cells in cambium region).

in the endodermis (fig. 4). In the central cylinder there were at low concentration relatively many cells of primary phloem and xylem but definite lignification was apparent only in the region of the protoxylem. The remainder of the stele consisted largely of small, closely packed, thin walled cells containing abundant protoplasm (fig. 4).

At low concentrations of nutrient at a point 5 cm. from the root tip (fig. 6) there were many root hairs, the cortical cells were somewhat more rounded and larger than at the earlier stage of development shown in figure 4, but there was no indication of death of the cortex. The central cylinder as shown in figure 6 (at 5 cm. from the root tip) was remarkable in that progress toward maturity had proceeded so slowly. Noticeable Casparian strips were lacking, there was not the slightest indication of fibers, and there was little lignified xylem. Many cells even of the metaxylem were still thin walled and, similar to the cells of the phloem and pericycle region, had rather dense protoplasmic contents.

The roots of the low concentration cultures at 15 cm. from the root tip exhibited in the region of the stelar cambium a remarkably wide band of thin walled cells containing relatively dense protoplasm (fig. 8). It will be recalled that, at the earlier stages described (figs. 4, 6), the growth of the roots at low concentrations included primary tissues that were slow in maturing, but practically no secondary elements. Nevertheless, at 15 cm. from the root tip there was an abundance of secondary phloem and xylem both of which matured slowly. At this stage the cortex, only a part of which is shown in figure 8, was sometimes more or less torn internally, although it was apparently alive and in external appearance smooth and white. The endodermis was usually intact and Casparian strips were clearly evident. Underlying the endodermis, there was some indication of a potential cork cambium, although during the period of the experiments there was practically no suberization of cell walls in that region and but comparatively slight thickening of the walls of the fibers.

In contrast, the plants receiving the higher concentrations of nutrient solution at 1 cm. from the root tip had few cortical cells. They were small and obviously rather mature in that they were rounded

and deficient in protoplasmic contents (fig. 5). Many root hairs were in evidence in this region, as were distinct Casparian strips. The elements composing the small central cylinder of the roots which de· veloped at higher nutrient concentrations (fig. 5) were also relatively mature. There were even present a few thick walled fibers over the primary phloem. All the elements of the primary xylem were strongly lignified and optically empty. There were a few secondary xylem and phloem elements owing to the early initiation of cambial activity. The secondary tissues of xylem and phloem, however, were extremely limited in amount and matured rapidly.

The roots of the high concentration cultures (fig. 7) had lost practically all cortical tissue at 5 cm. from the root tip, and from the region of the pericycle had arisen a cork cambium some of whose external derivatives had become strongly suberized. The roots were therefore rough and brown in external appearance as compared with those of the dilute nutrient solutions. Together with loss of the cortex and the appearance of a strong cork cambium, there were present many heavy walled fibers, and the bulk of the stele consisted of strongly lignified, optically empty secondary xylem elements. Most of the phloem tissue was also comparatively mature, and while the cambium was obviously active in the production of secondary tissue, the cells derived from it matured with extraordinary rapidity, leaving in consequence only a limited amount of embryonic tissue in the cambium region.

The roots which were produced in the cultures of high concentration presented much the same situation at 15 as at 5 cm. from the root tip (fig. 9). Fibers were somewhat thicker walled and there was a small increase in volume of secondary xylem and phloem, but the general characteristics were as already described.

The roots of the high concentration cultures have been characterized as woody, mechanically strong, and lacking in succulence. Associated with these conditions the well known fact may be recalled (16) that, although the intake of nutrients may occur freely with a fairly high concentration of salts in the nutrient solution (table V), there is a marked diminution in absorption of water. This factor undoubtedly contributed in part to the early loss of protoplasm and the rapid maturity of tissues when sweet pea was grown in a nutrient

medium of two or three atmospheres. This is in contrast with the comparatively slow maturation of the roots of the low concentration cultures.

It should be emphasized, however, that factors other than salt concentration may give very similar responses. For example, the anatomical responses of the roots of peach and apple (20) which were grown at a temperature of 75° F. (with the same nutrient solution as in these experiments, at a concentration of one atmosphere) corresponded in almost every detail with that of sweet peas as grown at two or three atmospheres at about 50° F. That the metabolic responses were also much the same will be shown later. Likewise peach and apple (20), with the nutrient treatment described above but at a temperature of 65° F., exhibited roots which resembled in anatomy and metabolism the roots of sweet pea which were supplied with the more dilute nutrient solutions.

STEMS.—In the preceding pages it has been shown that the lower concentrations of nutrient salts directly or indirectly resulted in the production of vigorously growing stems of comparatively large diameter; whereas with more concentrated solutions the stems grew less vigorously, were smaller in diameter, and comparatively woody (table II, fig. 2). The associated anatomical features are indicated in figures 10 to 15.

Even casual examination of these figures shows that the structure of the stems was influenced by the concentration of nutrient salts in a manner similar to that already recorded for the roots of sweet pea. It is obvious that the rate of maturity of tissues was much slower at low than at high concentrations of nutrient solution. At 1 cm. from the stem tip the plants of the more dilute solutions (fig. 10) exhibited in transverse section many cells which were practically all thin walled, closely packed, and filled with dense protoplasmic contents. Lignification was evident only in a few cells of the protoxylem, the metaxylem was extensive but thin walled, and there was only slight indication of cambial activity. In contrast, the stems of the cultures supplied with the more concentrated solution had at the same distance from the stem tip (fig. 11) much less primary tissue; and the cells, except in the region of the cambium and pericycle, were comparatively limited in content of opaque protoplasm. Further, the
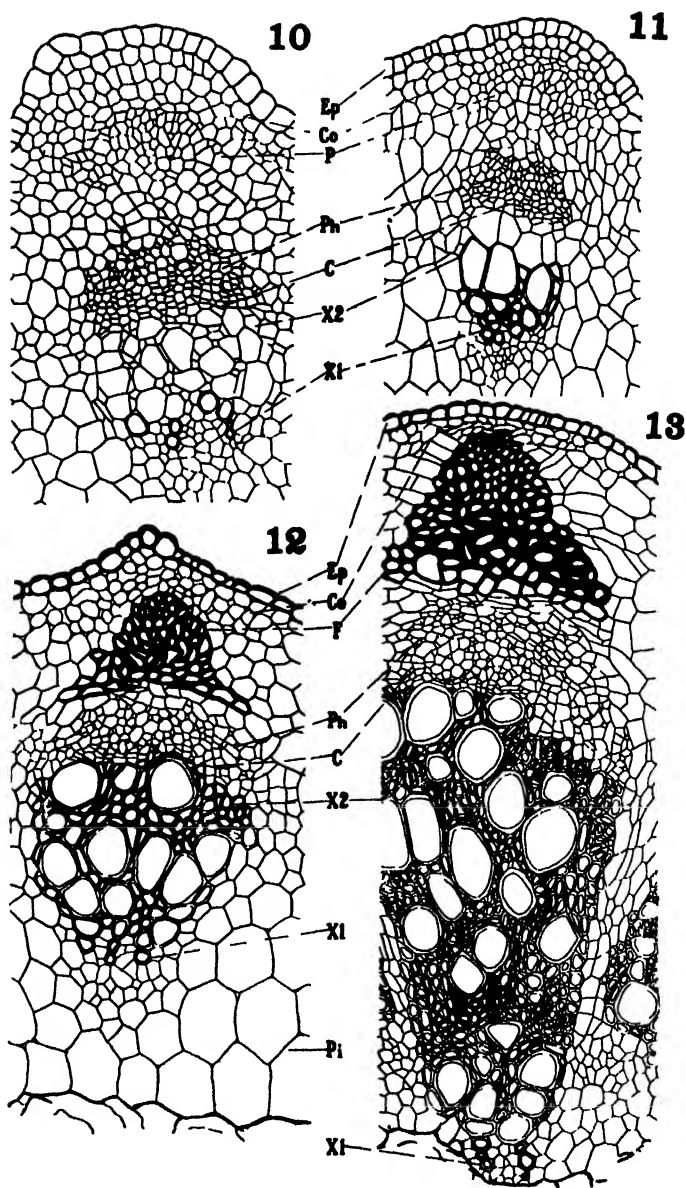
FIGS. 10–13.—Transverse sections of sweet pea stems. fig. 10, 1 cm. from tip, dilute nutrient solution; fig. 11, 1 cm. from tip, concentrated nutrient solution (note fewer primary cells and earlier differentiation); figs. 12, 13, concentrated nutrient solution, middle and base of stem respectively.
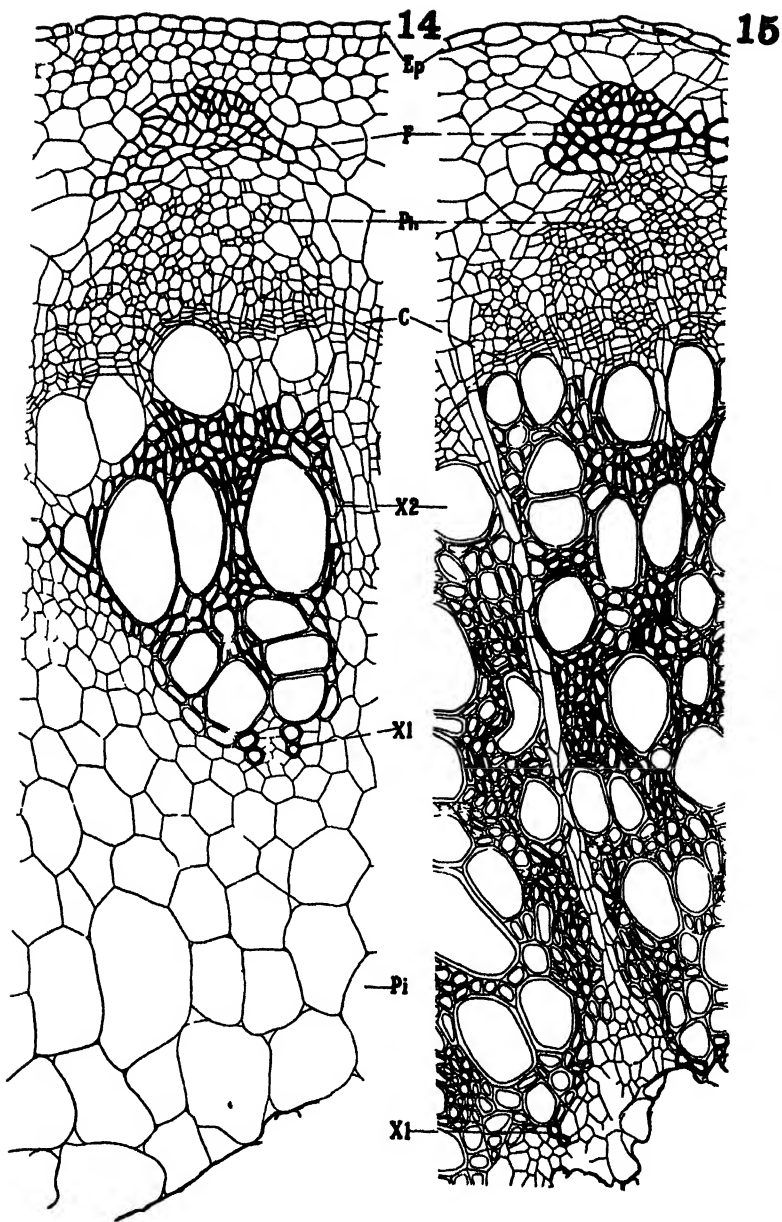
FIGS. 14, 15.—Transverse sections of sweet pea stems, dilute nutrient solution middle and base of stem respectively. Compare with figures 12 and 13 which show earlier maturity of tissues, heavier fibers, and limited cambium region with concentrated nutrient solution.

primary vascular elements, although much less in total volume than at lower concentration of nutrients, were strongly lignified and optically empty in both protoxylem and metaxylem regions, and the cells of the phloem were comparatively limited in content of protoplasm. Likewise in accord with the rapid differentiation of primary tissues there was apparently present some secondary phloem and xylem, the latter rapidly undergoing the early stages of lignification and loss of cell contents.

Transverse sections of typical vascular bundles (figs. 12, 14) at about the middle of the respective primary stems yielded information strictly in harmony with the preceding observations. The plants supplied with the higher concentrations of nutrient salts showed that there had been not only more rapid maturity of primary elements, including loss of much of the pith, but that there was rapid maturity of secondary tissues as well. Associated with the more dilute nutrient solutions many more pith cells persisted and exhibited protoplasmic contents, and there was much more extensive development of secondary xylem and phloem, the cell walls of the former becoming lignified and losing their cell contents slowly, even the oldest cells of the phloem showing denser protoplasm. The slow differentiation of secondary elements necessarily left in the cambium region a remarkably wide band of young, thin walled cells (fig. 14). In the region of the pericycle there were in evidence only the early stages of development of fibers.

Fibers of the groups at high concentration of nutrients, however, were very thick walled (fig. 12), the cortical cells more rounded, and the epidermal cells and cuticle considerably thickened. Secondary phloem and xylem matured so rapidly that there was in transverse section but a narrow band of cells in the cambium region. All these factors contributed to the relatively small diameter and lack of succulence of the stems of the sweet pea plants which were supplied with the higher concentrations of nutrient solution.

Drawings were also made (figs. 13, 15) of transverse sections of the base of the stem just above the transition region of exarch and endarch xylem (13). The stems at this point presented a situation in accord with that exhibited by the middle of the stem. Owing to a very active cambium there was much more secondary xylem and phloem

and a correspondingly thicker stem at low than at high concentrations of nutrient, but pericycle fibers in the former were limited in number and in thickness, and in the region of the cambium there were many actively dividing cells containing dense protoplasm. In clear cut contrast, the base of the stems of the cultures of higher salt concentration (fig. 13) showed large bundles of strongly developed pericycle fibers, and phloem and xylem which were much less extensive owing to less cambial activity. In fact in the entire transverse section there were few cells that were not optically empty and, in the region of the cambium, mature phloem and strongly lignified xylem elements frequently abutted, one upon the other.

It is obvious that these anatomical features were intimately associated with the external growth responses already recorded, the condition of woodiness and lack of succulence at higher concentration of nutrients, and the comparatively soft and extremely succulent growth of stems of the plants supplied with the more dilute solution of nutrient salts.

LEAVES.—The anatomical structure of the thin, dark green, succulent leaves of the plants supplied with dilute nutrient solutions is indicated in figure 16. This may be compared with that of the cultures of higher nutrient concentrations (fig. 17) which, it will be recalled, bore leaves that were smaller in area, lighter green, thicker, and much less succulent (fig. 3). The associated anatomical differences are clearly in harmony with the external appearance of these leaves.

At high concentration of nutrients the cuticle of the upper epidermis was heavy, the palisade mesophyll distinct, and the spongy mesophyll relatively compact. In contrast, the plants supplied with more dilute nutrients exhibited leaves in which the spongy mesophyll was prevalent and loosely arranged, in that there were larger and more numerous intercellular spaces. The somewhat smaller size of mesophyll cells in the latter group, however, is at variance with the relative size of cells in roots or stems of corresponding series. It will be remembered that these organs had cells which were notably large in the plants of the low as compared with those of the high concentration cultures. Why the blades failed to present a corresponding situation is not certain. It may be mentioned, however, that the per-

centage of sugars and starch was extremely low in the blades of the plants which received the more dilute nutrient solutions (table V). The low content of sugars may have been intimately associated with the osmotic concentration of the cells concerned and consequently with cell expansion. In the stems and roots of the same group of
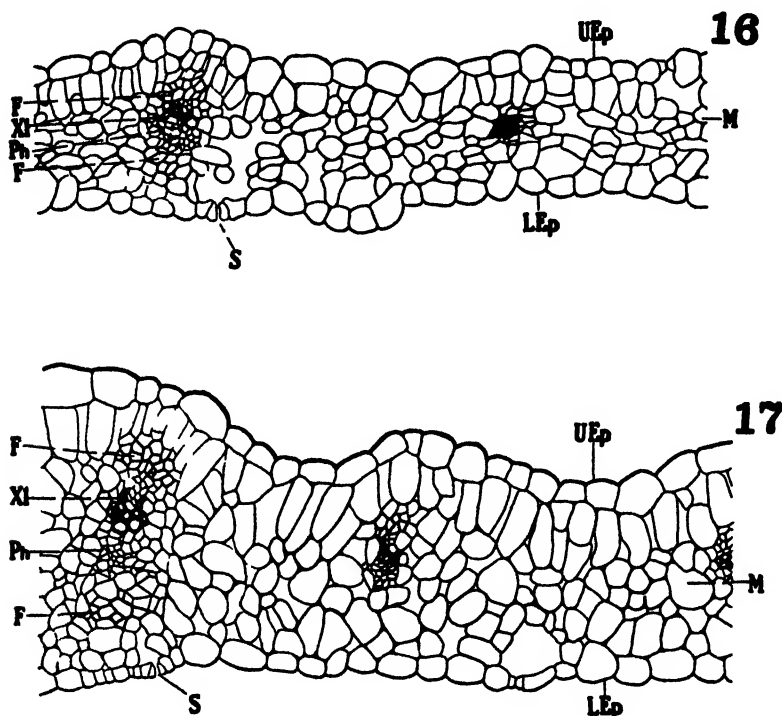
FIGS 16, 17 —Transverse sections of sweet pea leaves, dilute and concentrated nutrient solution respectively Note relatively xerophytic structure of leaf from plant supplied with concentrated nutrient solution

plants, correlated with a high water content but less drastic reduction in percentage of sugars and starch, there was found the comparatively large size of cells and slow rate of maturation of tissues already mentioned. It should be emphasized, however, that, as in other organs of the plants, the blades of the cultures supplied with the more dilute solution of nutrient salts appeared much less vacuolated and contained larger and apparently darker green chloroplasts

than the plants which received the more concentrated nutrient solutions.

Between the two groups there was little if any difference in the number of stomata found in corresponding areas of mature leaves. Repeated measurements demonstrated, however, that the veins were much more numerous per unit area in the blades of the high concentration cultures; further, the bundles of fibers of the veins were larger and thicker walled (fig. 17). Frequently veinlike bundles were found in which only fibers were clearly evident.

The preceding descriptions are obviously not specific as to effects of the respective salt concentrations of the nutrient medium. The blades of the high concentration groups are similar in external appearance and anatomy to so-called "sun leaves" or to leaves which develop under xerophytic conditions including a limited supply of soil or atmospheric moisture or both (16, 24). Nevertheless such a response would be anticipated, as it is well known that with increase in concentration of salts in the nutrient or soil solution there is a marked diminution in rate of water absorption (16). Conversely with a more dilute solution of salts there is greater intake of water. The blades of the sweet pea plants with the less concentrated nutrient solutions exhibited the typical characteristics of plants in a fertile soil with abundant moisture, or of plants grown at high humidity or in the shade where likewise the percentage of dry matter in the plant is low but the percentage of water high (15, 16, 24).

ABSCISSION ZONE.—The structure of the peduncle and pedicel of sweet pea was found to be similar to that of the stem except that cambial activity apparently ceased at about the time buds abscissed or blossoms matured. The anatomical structure of the flower stalk and pedicels was affected by the concentrations of nutrient salts employed in essentially the same way as were the stems. It will therefore be unnecessary to present a detailed anatomical description of these plant parts.

As shown in table II, in neither nutrient series did all of the flower buds absciss. At low concentration of salts, these peduncles that bore flowers to maturity were of comparatively large diameter and succulent, owing, as in case of stems of the same group, to relatively many primary cells, greater cambial activity, and larger cells of both

primary and secondary tissues, both of which differentiated and matured slowly. Following or accompanying a period of cloudy dark days, however, the peduncles and pedicels became more succulent, maturation became even slower, and from the plants of the low concentration cultures there was frequently nearly 100 per cent abscission of buds, separation occurring at the point of attachment of the pedicel to the main flower stalk.

At this point, the peduncle and pedicel of such abscissing buds were typically of small diameter, as illustrated in figure 18, which shows an approximately median longitudinal section through the potential abscission zone eight days after the appearance of a bud. This bud, typical of many others of the same group of cultures, was obviously going to absciss as evidenced by the premature straightening of the peduncle (fig. 3). It was borne on a pedicel remarkable for its immaturity. With the exception of a few primary xylem vessels, there was no evidence of lignification and fibers were absent. Throughout much of the pedicel, even in cortex and pith, dense cytoplasm and large nuclei prevailed together with a high concentration of amino acids and proteins.

In the region of the potential abscission zone at the approximate level of the grooved ring of the cortex (fig. 18), there was clearly evident tissue ten to fifteen cells in thickness that was delimited by the smaller size of its cells and their very dense contents. It extended transversely through the pedicel, and with the exception of the primary xylem vessels practically all cells at this level exhibited the condition described. At this stage the cells of this potential abscission layer were not noticeably different from neighboring cells, above and below, in content of mineral nutrients. Reducing sugars and sucrose were present but practically no starch. As already mentioned, the protoplasmic content of the pedicels was high and particularly so in the cells of the zone of abscission. After denaturization with dilute alkali, the contents of such cells gave strong protein reactions.

The material for figure 19 was obtained from a plant of the series which received the highest concentration of nutrient salts (table I) and from a pedicel typical of many others of that group of plants. The pedicel was bearing a bud which presumably would not have ab-
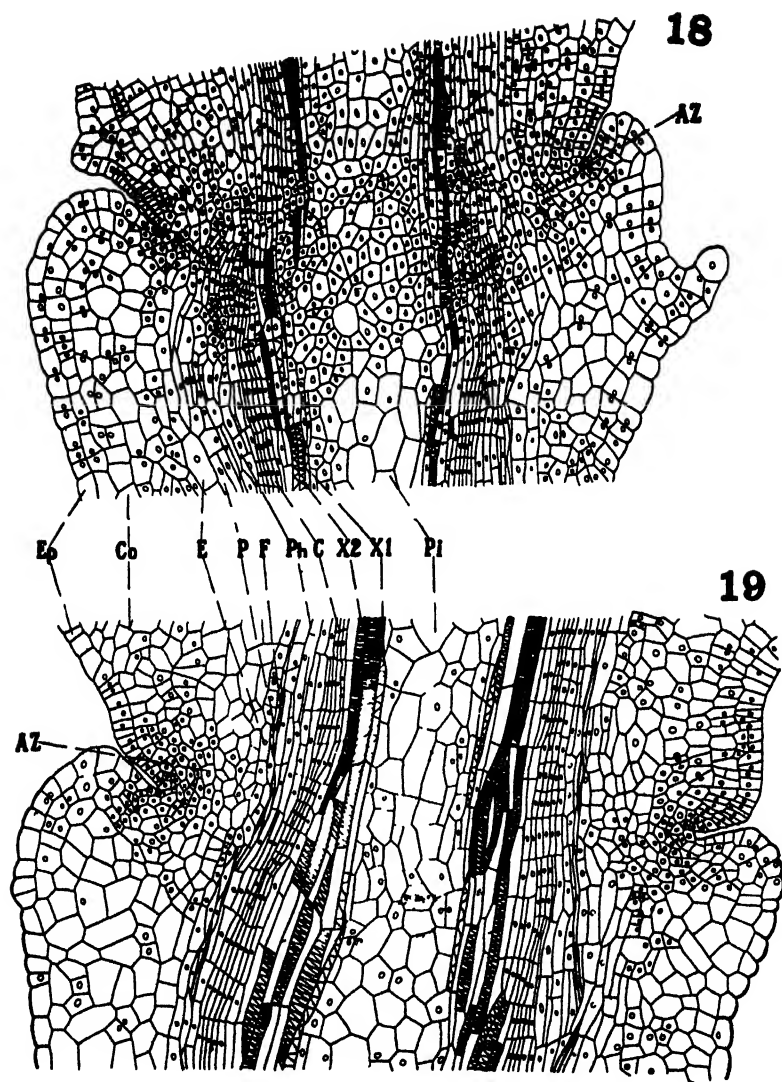
FIGS 18, 19 —Longitudinal sections through pedicel and peduncle of sweet pea in region of abscission zone 8 days after bud was evident, dilute and concentrated nutrient solution respectively. Note that with dilute nutrient solution differentiation and maturity of tissues were slow. Several tiers of meristematic cells in region of potential abscission zone (fig. 18).

scissed, as indicated by the condition of the peduncle which was downwardly curved (fig. 3). Further, the peduncle and pedicel were relatively woody and lacking in succulence.

Figures 18 and 19 are comparable in the sense that both drawings were made from material harvested eight days after a bud had appeared on the pedicel concerned. However, the similarity ends there. The material, although comparable in age as expressed in days, was strikingly dissimilar in degree of maturity. The peduncles and pedicels of the cultures of high nutrient concentration were much more mature in every way. The pith cells, although not yet fully enlarged, were comparatively free of protoplasm; both primary and secondary xylem were strongly lignified, and there was relatively much of the latter due to early production of secondary tissues. Sieve and companion cells were clearly differentiated, and bordering these elements there were present thick walled fibers in the region of the pericycle. In the cortex, except near the potential zone of abscission (cortical groove or junction point of peduncle and pedicel, figure 19), all cells were much vacuolated and largely devoid of nuclei. Sugars and starch were, however, notably high in all parenchymatous cells.

Four days later, comparable buds borne on the plants supplied with the dilute nutrient solution had abscissed. Immediately after abscission, the separation layer and remaining peduncle exhibited the situation indicated in figure 20. The cells of the separation layer failed to mature and showed all the characteristics of immature cells. They were comparatively small and angular and displayed dense cytoplasm and large nuclei; they contained an abundance of proteinaceous constituents, sugars, minerals, and nitrate. No deficiency of calcium in the protoplasm was noted, although this element was apparently present in large part in combination with proteins or other materials and was found in greatest abundance only following denaturization of proteins with alkali as described elsewhere (21). However, this seemed to be generally true of all meristematic tissues in sweet pea.

Accompanying foliar abscission of *Coleus*, SAMPSON (29) reported a deficiency of calcium in the cells of the separation layer and suggested that this may have an important bearing on the degree of solidity of the so-called calcium pectate middle lamella. In sweet pea,
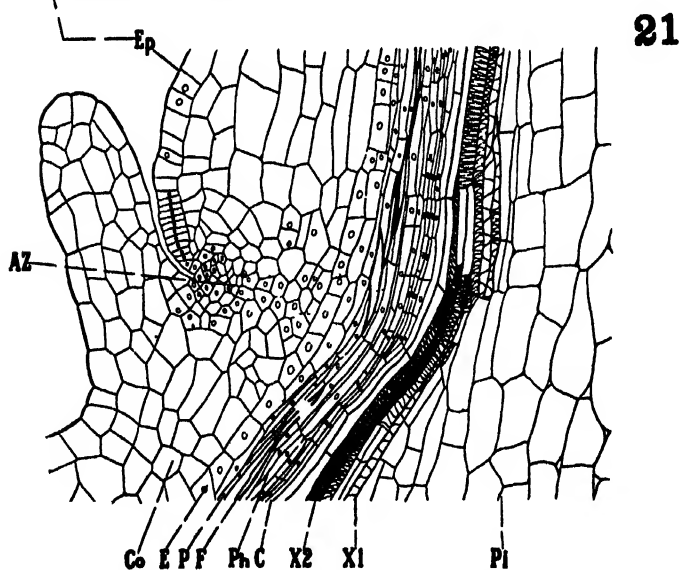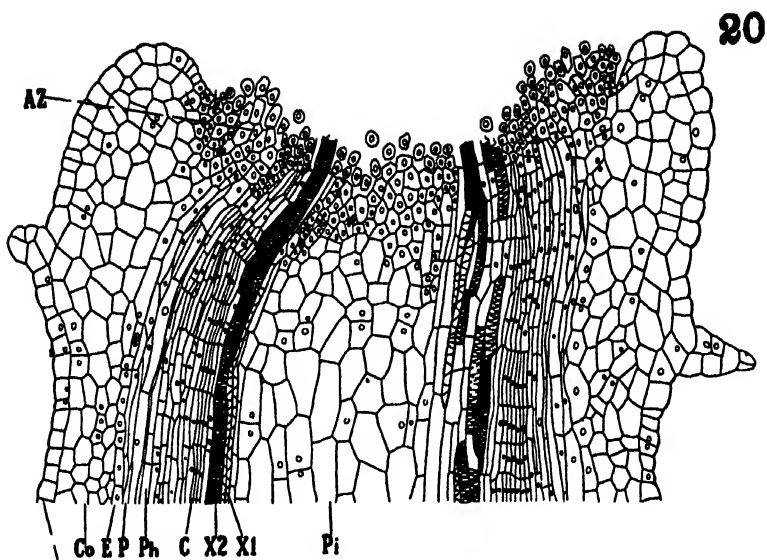
FIGS. 20, 21.—Longitudinal sections through pedicel and peduncle of sweet pea in region of abscission zone 12 days after bud was evident, dilute and concentrated nutrient solution respectively. Fig. 20, pedicel and bud abscissed. Note cells which failed to mature in region of abscission zone. These cells became rounded and with gelatinization of middle lamella there occurred separation of pedicel from peduncle. Fig. 21, tissues mature and highly differentiated. Only half of section shown.

with the nutritional treatments here employed, there seemed to be no lack of calcium in the cells of the abscission zone nor in bordering tissues.

The actual separation of cells in the abscission layer of sweet pea pedicels was preceded by some swelling and gelatinization of the pectic constituents of the middle lamella. Although not so pronounced as in foliar abscission in *Citrus* (14), abscission was of the conventional type, involving dissolution of the middle lamella as described by HODGSON (14) and SAMPSON (29). The cells so freed from neighboring tissues were round, very thin walled, and seemed still alive immediately after separation from the other cells. At least they were similar in appearance to formerly adjacent cells of analogous intact tissue except that they contained starch which, at the time of their separation, was practically absent from all other cells except those of the endodermis.

Starch began to appear in the cells of the separation layer at about the time of swelling of the middle lamella. It may very well have been a secondary effect, and HODGSON, who found a similar situation in *Citrus*, suggests that "changes occur in the cell walls of the abscission zone which prevent the translocation of the products of starch hydrolysis to adjacent cells" (14).

Separation did not follow a right-angle plane across the main axis of the pedicel but varied apparently according to the mechanical resistance encountered. The number of tiers of cells actually taking part in abscission varied from one or two to several. Abscission usually began in the cortex in the general vicinity of the grooved ring and proceeded inward, involving all tissues except lignified xylem elements. No pericycle fibers were present in the soft succulent peduncles and pedicels of the buds which abscissed.

The peduncle below the zone of abscission (fig. 20) had matured considerably during the four-day interval, but there was nevertheless little lignified xylem and no pericycle fibers. Pith and cortical cells were angular, closely packed, and characteristically contained considerable cytoplasm and frequently nuclei.

Following abscission, the peduncle rapidly accumulated an enormously high concentration of sugars, starch, amino acids, and mineral elements, which presumably were being carried to the develop-

ing bud prior to loss of that organ. The flower stalks of inflorescences which had abscissed persisted on the plants for the duration of these experiments, and, with the accumulation of foods as already described, lost their initial succulent condition and became woody and increased somewhat in diameter.

The material for figure 21 was obtained from a culture of the series receiving a high concentration of nutrient salts and from a typical pedicel which was clearly not going to absciss and which bore a bud that was far enough advanced just to show the color of the emerging petals. The flower stalk was harvested for anatomical study twelve days after the bud first appeared, four days later than the material of the same series in figure 19. As expressed in time it was of the same age as the peduncle from which the bud abscissed (fig. 20).

There has already been described the comparatively immature succulent condition of the peduncles and pedicels of the latter group and the general process of abscission. Associated with the woody, non-succulent condition of the pedicel and persistence of buds of the former series, there was found only a few cells which were immature in character. These cells, occurring in the potential abscission zone in the vicinity of the cortical ring or groove (fig. 21), were small, angular, and thin walled, contained dense cytoplasm and large nuclei and were practically free of starch. Distal and proximal to this region the cells of the cortex were abruptly much larger, more vacuolated, and high in content of starch. Pericycle fibers were strongly developed and cambial activity gave rise to phloem and heavily lignified xylem elements. The pith cells and primary xylem parenchyma were also mature and much vacuolated, but high in concentration of starch and nitrate. Amino acids and asparagine were present in considerable quantities but were not so abundant as in case of the more succulent series of the low concentration cultures.

Abscission of the flower buds of sweet pea was therefore intimately associated with a deficiency of carbohydrates, and as usual (15, 18, 27, 28, 34) under such conditions tissues matured slowly and were lacking in mechanical strength. In the very young pedicels there was, of course, no apparent way of distinguishing the tiers of cells which were destined to form the abscission layer, as all tissue was

relatively meristematic. But in case of peduncles and pedicels which were low in reserve carbohydrates and obviously going to drop their buds, no subsequent or older stages could be found in which young angular cells with dense contents were not present in the region of the potential zone of abscission. On the other hand, the cultures of the series which received a high concentration of nutrient salts and which were higher in carbohydrates matured rapidly and did not exhibit this condition (figs. 20, 21).

The mode of derivation of the abscission zone already described is in contrast to the abscission through the non-succulent pedicels of mature fruits or the abscission through the comparatively woody petioles of mature leaves. For example, SAMPSON (29) found that in the case of the foliar abscission of *Coleus* mature cells became active and divided and thus gave rise to tiers of meristematic cells involving all tissues except the lignified xylem elements.

There have been recorded in the preceding pages effects of the salt concentrations of the nutrient solution on the anatomical structure of the vegetative organs of sweet pea. Similar effects are also described for peduncle and pedicel, together with discussions concerning the structure and composition of these organs in their relationship to premature abscission of flower buds. In the following pages it is proposed to show that certain phases of metabolism were intimately associated with the responses already recorded.

ABSORPTION AND ASSIMILATION OF NITRATE

The sweet pea plants of these experiments contained an abundance of nitrate in roots, stems, and leaves. The various concentrations of salts employed under the seasonal conditions of these experiments were all such as to furnish the plants with more than an adequate supply of nitrate for vigorous protein synthesis. With the microchemical observations, the total ash determinations (table V) also gave evidence indicating that mineral elements were not lacking in the tissues of the plants of the respective series. In general the nutrient solutions of higher salt concentrations resulted in a definitely higher percentage of nitrate in all organs of the plants concerned (table V), as compared with those supplied with more dilute nutrient solutions. As will be shown in the following pages, however, this was

probably due in part to limited utilization of nitrate in protein synthesis, with consequent accumulation of the unelaborated nitrogenous nutrient. EMMERT and BALL (11) also found that with decrease in moisture content of soils there was increase in the percentage of nitrate found in the tissue of tomato plants.

Whereas the several concentrations of salts of the various nutrient treatments permitted adequate absorption of nitrate and other nutrients, they indirectly influenced very greatly the capacity of the roots and tops to reduce nitrate to nitrite, ammonium, and amino acids. This process of reduction and synthesis has been termed *nitrate assimilation* (20). The relative rate at which this phase of protein synthesis takes place is indicated with considerable accuracy by ECKERSON'S method (7, 20), which "consists of taking an aqueous extract of fresh plant tissue and measuring the amount of nitrite reduced from nitrate by a given sample under specific conditions of time, pH, and temperature. The amount of nitrite formed from nitrate gives a measure of the *reducase* activity of the particular plant or organ sampled." Analyses of plant material (9, 18, 20, 22) have demonstrated that reducase activity closely parallels the synthesis in the plant of amino acids and other forms of organic nitrogen. Reducase activity therefore makes available a convenient index of the relative rate of nitrate assimilation.

The results of such determinations are shown in table IV. The roots of sweet pea were consistently low in ability to assimilate nitrate, as compared with perennial plants such as apple (8, 20, 22), asparagus (26), and narcissus (25); but as in the case of apple (20), it was the young succulent tips which were most active in nitrate reduction, not the older roots which were less succulent and contained much less protoplasm. The roots of the plants of the cultures receiving the more dilute nutrient solutions (one half or one atmosphere) were higher in reducase activity. These results were not surprising, however, as it will be recalled that these roots were slow in maturing; the cortex persisted and many cells retained relatively dense protoplasmic contents for a considerable period. The roots of the cultures supplied with the more concentrated solutions of salts lost their cortex early, and in the central cylinder, cells containing dense protoplasm were few. The prevailing elements of the stele were mechani-

cal fibers and strongly lignified xylem (figs. 3–9). Such tissues are notably lacking in ability to synthesize proteins from nitrate (20).

That a similar situation prevailed in the stems is also clear (table IV). The most vigorous reduction of nitrate occurred in the younger portions of the stem where there was present a higher proportion of cells containing protoplasm. Proceeding from the tip to the base of

TABLE IV

REDUCTION OF NITRATE BY EXTRACTS OF DIFFERENT PORTIONS OF SWEET PEA PLANTS (SAMPLES FROM MAIN AXIS; STEM TIP TO ROOT TIP AT POINTS INDICATED)

	APPROXIMATE CONCENTRATION OF NUTRIENT SOLUTION IN ATMOSPHERES							
	DEC. 8, 1934				JAN. 4, 1935		JAN. 7, 1935	
	½	1	2	3	½	3	½	3
	NITROGEN (MG.) AS NITRITE PER GRAM FRESH WEIGHT							
Distance from stem tip (cm.)								
Stem tip (first 30 cm.)..	0.018	0.020	0.013	0.010	0.047	0 019	0 032	0.012
90	0.027	0.031	0 005	0 009	0.036	0 012	0 030	0.010
150	0.015	0 011	0.001	0.002	0.015	0 007	0.007	None
210	0.009	0.008	None*	None*	0.009	Trace	0 006	None
270	0.007*	0.007*	......	......	0.001	None*	Trace	None*
280	......	......	......	......	None*	......	None*	.....
Old roots (diameter 4 mm.)	Trace	None	None	None	None	None	None	None
Old roots (diameter 2 mm.)	0 001	0 001	None	Trace	0 001	Trace	0.001	Trace
Root tips (1–2 cm. in length)	0.004	0.003	0.001	0.001	0.003	0.001	0 002	0.001

* Base of stem

the stem, there was a marked decrease in rate of assimilation of nitrate in all groups of plants. But this decrease was much more marked in the stems of the cultures supplied with the more concentrated nutrient solutions (two and three atmospheres respectively), and quite logically so, as the anatomical studies have shown (figs. 10–15) that there was much more rapid maturity of cells. This resulted in tissues which were strongly developed mechanically but relatively low in proportion of cells containing abundant protoplasm.

Even the sample termed *stem tip*, which included the distal 30 cm. of linear stem growth, was on any given date much lower in reducase activity in the cultures of high nutrient concentration than in those supplied with more dilute salt solutions (table IV). As already shown, however, even at 1 cm from the tip the stems of the former cultures were further advanced in maturity and relatively low in proportion of opaque protoplasm; consequently they were low in reducase activity.

The matter of variation in reducase activity on different dates is probably associated directly or indirectly with the seasonal light conditions. In tomato at least (9), cloudy weather or long nights (short days) definitely decreased the reducase activity. The reason for this is not known, but it should be recorded that there was much more cloudy weather (and of course the nights were longer) in early December preceding the harvest of plants than later in the month just before the determinations were made in early January.

However, although cells lacking abundant protoplasm are low in nitrate reducing capacity, it seems also to be true that tissues are limited in ability to assimilate nitrate if carbohydrates are extremely deficient (6, 9, 18, 20) (Obviously reduction of nitrate cannot occur without oxidation of carbohydrates or their derivatives) It is perhaps significant that the sample designated as *stem tip* of the series of low nutrient concentration was definitely low in sugars when harvested on December 8, and this is the only case where the distal sample of stem tissue was lower in reducase activity than the portion of older tissue of the stem 150 cm from the tip (table IV). But the latter sample was much higher in carbohydrate content The stem tip samples of the cultures supplied with more concentrated nutrient solutions were much higher in sugars and fluctuated relatively little in nitrate reducing ability.

The results discussed in the foregoing paragraphs would not seem to indicate that the higher concentrations of salts employed directly limited the synthesis of proteins from nitrate. Rather, a nutrient medium high in concentration of salts is known to limit the absorption of water (16). As already recorded, the plants exhibited in external appearance and anatomy responses which were relatively xerophytic in nature. The plants were strongly developed mechani-

cally but lacking in succulence, and contained comparatively few cells with dense protoplasmic contents, a characteristic which is essential for vigorous assimilation of nitrate.

CHEMICAL COMPOSITION

NITROGENOUS FRACTIONS.—It has already been pointed out that the percentage of nitrate in roots, stems, and leaves was directly correlated with the concentrations of this material in the nutrient solution. The fact that there were different amounts of nitrate in the several series of plants was probably of little significance in relation to protein synthesis, as there was present in all cases an adequate amount for this phase of metabolism. Nitrate is not an essential part of the living protoplasm but represents an excess of nutrient material not yet assimilated (25, 26, 27).

That the rate of synthesis from nitrate of amide, amino, and protein nitrogen was relatively rapid in the plants supplied with the more dilute nutrient solutions has already been indicated by the reducase determinations (table IV), and is further corroborated by the results of macrochemical analysis (table V).

In all parts of the plants, the percentage of total assimilated or nitrate-free nitrogen tended to be consistently higher in the series supplied with the dilute nutrient solutions (one half and one atmosphere) than in those grown with a greater concentration of salts in the nutrient medium (two and three atmospheres). Also there was obviously a much greater absolute amount of elaborated nitrogenous material in the plants grown in the less concentrated solutions, for they were greater in volume (fig. 1) and total green weight (table II). Further, the percentage of ammonium, amino, and amide nitrogen was notably high in all parts of these plants. But this would seem reasonable, as reducase activity was also much higher in these cultures (table IV). In the assimilation of nitrate and synthesis of proteins, the nitrogenous materials mentioned would be among the first products formed and would tend to be present in greatest amount in plants which were most active in this process.

Associated with the comparatively high percentage of these simpler water soluble forms of organic nitrogen, the more complex protein fraction was relatively low (table V) in the plants grown at the

more dilute nutrient concentrations of one half and one atmosphere. This is, however, a situation commonly found in plants that are succulent and growing vigorously (5, 12, 17, 25, 26, 27, 28, 32). Proteinaceous material is presumably high in meristematic tissue, but storage proteins do not seem to accumulate in tissues that are low in dry matter. Although protein constituted a comparatively high proportion of the total organic nitrogen of the plants supplied with the

TABLE V

NITROGENOUS AND CARBOHYDRATE FRACTIONS, ASH, AND DRY MATTER
IN VEGETATIVE ORGANS OF SWEET PEA, DECEMBER 9, 1934, EX-
PRESSED AS PERCENTAGE OF GREEN MATTER

	APPROXIMATE CONCENTRATION OF NUTRIENT SOLUTION IN ATMOSPHERES											
	STEMS AND PETIOLES				BLADES				ROOTS*			
	½	1	2	3	½	1	2	3	½	1	2	3
Total nitrate-free N	0.431	0 411	0 354	0.379	0 647	0.620	0.612	0.600	0.142	0.140	0.130	0.118
Protein N	0 236	0.211	0.265	0.304	0 507	0 500	0 539	0.546	0 070	0 079	0.097	0.094
Nitrate-free soluble N	0.195	0.200	0.089	0 075	0.140	0 120	0.073	0.054	0.072	0.061	0 033	0.024
a-amino N	0.052	0 057	0.017	0.025	0.032	0.040	0.020	0 011	0.031	0 022	0 002	0.002
Amide N	0.040	0.051	0.020	0 019	0 020	0 028	0.015	0 014	0 011	0.017	Trace	Trace
Ammonium N	0.004	0.006	0.001	0 002	Trace	Trace	Trace	Trace	0.002	0 003	Trace	Trace
Nitrate N	0.020	0.032	0.058	0 083	Trace	0.007	0 042	0.036	0 010	0 058	0 123	0.182
Total N	0.451	0.443	0.412	0.462	0 647	0 627	0 654	0 636	0.152	0.198	0.253	0.300
Dry matter	15 00	13.60	18 40	18 00	14 00	13.40	17.00	17.00	5 70	6 60	7.90	8.00
Reducing sugars	0 77	1.30	1 41	1.53	0.30	0.26	0 91	1.00	0.70	0.31	1.36	1.27
Sucrose	Trace	0 17	0 15	0.27	0.12	0 05	0.29	0.39	0.00	0.16	0.39	0.74
Total sugars	0.77	1.47	1.56	1 80	0 42	0 31	1.20	1.39	0.70	0.47	1.75	2.01
Starch and dextrin	0 39	0.49	0 81	0.79	0 55	0 63	1.47	1 53	0 28	0.37	0.42	0.31
Total carbohydrates	1 16	1.96	2.37	2.59	0 97	0 94	2.67	2 92	0.98	0.84	2.17	2.32
Ash	0.15	0 14	0.16	0.17	0.18	0 17	0.21	0.22	.....	.....	.....	.....

* Results expressed as percentage of ash-free green matter.

more concentrated nutrient solutions (two and three atmospheres), it will be shown presently that these plants were relatively high in dry matter, including sugars and starch, and low in percentage of moisture. The later stages of the synthesis of proteins, at least of the storage type, presumably involves chemical dehydration, as in the condensation of amino acids to polypeptides. In plant organs, it has frequently been found that when amino acids and similar compounds are available, the synthesis of storage proteins occurs with decrease in percentage of moisture and increase in concentration of carbohydrates in the plant. It is not a result peculiar to effects of high salt concentration in the nutrient medium, however, for many factors of

nutrition and environment may be employed to give an increase in percentage of dry matter and decrease in moisture (5, 12, 17, 25, 26, 27, 28, 32, 35).

It may be noted, also, that, as compared with stems, the blades and roots of sweet pea have a very high proportion of their total elaborated nitrogen in a relatively complex protein form (table V). This is not in any sense peculiar to the cultures of these experiments, however, but seems to be generally true of other plants (4, 26, 27, 32).

CARBOHYDRATES.—The results of the carbohydrate analyses are shown in table V. Detailed comments would seem unnecessary. Practically without exception, reducing sugars, sucrose, and starch are much higher in all parts of the plants of the less succulent series which were supplied with the comparatively concentrated nutrient solutions (two and three atmospheres).

The relative rate of carbon dioxide exchange in the several series of cultures is unknown. Such determinations would have added materially to the value of the experiments. Nevertheless in the reduction and assimilation of nitrate, there necessarily occurs oxidation of carbohydrates or their derivatives, resulting in a decrease of those in storage unless supplied by new synthesis. The comparatively rapid reduction of nitrate (table IV) by the plants of the cultures supplied with the dilute nutrient solutions (one half and one atmosphere) undoubtedly contributed to the relatively low concentration of carbohydrates found in the tissues of these plants.

General discussion

These experiments on effects of the salt concentration of the nutrient medium were conducted with sweet pea but it would seem reasonable to suppose that other kinds of plants, although varying in degree of response, would react in a somewhat similar manner. There appear to be frequent demonstrations of this fact in the field and greenhouse. As pointed out in the introduction, the more successful growers of greenhouse plants water very sparingly during the winter, when opportunity for carbohydrate synthesis is limited. By so doing they obviously increase the salt concentration of the soil solution, and thereby limiting the absorption of water, obtain early maturity of tissues and a decreased rate of protein synthesis from

nitrate. Carbohydrates tend to be conserved, therefore, and to accumulate as they are used less vigorously in protein manufacture.

The employment of a high salt concentration is in practical effect that of a continuous but low plane of nitrogen nutrition, and plants exhibit the typical characteristics of such treatment. Clearly when opportunity for carbohydrate synthesis improves, as in the longer brighter days of late winter and early spring, the concentration of salts in the soil solution should be diluted by more copious watering. The more successful growers do just this. The continued employment of a high salt concentration in the long bright days of spring resulted in marked symptoms of nitrogen deficiency in a few of the sweet pea plants of these experiments which were allowed to remain after the investigation was discontinued. Plants of the low concentration cultures during the same period became much less succulent and carbohydrates accumulated. They became very similar in character to the high concentration series of midwinter. It would seem that to obtain ideal vegetative and reproductive growth, the protein nutrition of plants must be considered in relation to opportunity for carbohydrate synthesis. By varying the salt concentration with seasonal light conditions through judicious watering of soil-grown plants in the greenhouse, it seems probable that there may be controlled in considerable degree the rate of protein synthesis and quality of growth of plants.

Soil scientists have correctly emphasized the fact that plants can obtain water over a wide range of soil moisture content from the maximum field capacity to nearly the wilting coefficient (2, 33). But, as has been shown in these experiments, a high salt concentration may have marked effect on plant growth and metabolism even though salts are not sufficiently concentrated to result in plasmolysis of the root hairs or wilting of the leaves. In an apple or peach orchard, for example, effects of a low supply of moisture in the soil (high salt concentration) would be very drastic long before the moisture supply became low enough so that the leaves would wilt. In apple (8, 19, 22), peach (3, 23), and in many of our perennial plants (9, 25, 26), probably including certain grasses (30), the initial stages of protein synthesis occur almost exclusively in the fine suc-

culent rootlets; but in a solution high in concentration of salts, roots rapidly become woody, optically empty, and lose their capacity for protein synthesis. Nitrate absorption is not limited but the roots lack the capacity to assimilate nitrate, that is, synthesize amino acids and proteins. The effect will therefore be that of "nitrogen deficiency," or more accurately, that of protein deficiency.

Although somewhat aside from the subject of this paper, it may be pertinent to point out that long before the wilting coefficient of the soil is reached, there will be not only an increase in total concentration of salts, but with limited moisture some salts will presumably go out of the soil solution sooner than others. EMMERT and BALL (11) found that dry soil caused plants to accumulate in their tissues high concentrations of nitrate, but a reduction of moisture in the soil was associated with a decrease in percentage of phosphate.

Calcium phosphate might well be one of the first nutrient materials to go out of solution in a soil that was low in moisture content. But under most circumstances (21) effects of calcium deficiency occur early and are more severe than effects of insufficient phosphate (7). In New Jersey, apple orchards have frequently been found which exhibited roots that were high in nitrate but which were definitely deficient in calcium, as shown by external symptoms and analyses. Following an increase in water content of the soil, calcium was apparently again brought into solution; at least newly developed roots exhibited no sign of calcium deficiency and contained an abundance of this element.

Summary

In the fall and early winter of 1934, sweet pea plants were grown under usual commercial conditions of temperature and humidity. All of the cultures received a complete nutrient solution which was continuously percolated through the white quartz sand of the self-draining porcelain culture jars. Each group of plants received an abundant supply of nitrogen as nitrate and the same ratio of nutrient salts. Some of the plants were supplied with relatively dilute nutrient solution at concentrations of one half and one atmosphere respectively, others with comparatively concentrated solution at two and three atmospheres respectively.

1. The plants supplied with the more dilute nutrient solutions grew vigorously and were relatively succulent; the leaf blades were thin, somewhat circular in shape, and dark green. A comparatively high percentage of flower buds abscissed.

2. The cultures supplied with the more concentrated nutrient solutions grew less vigorously and were less succulent; the leaf blades were thick, oval or eliptical in shape, and light green. A comparatively low percentage of flower buds abscissed.

3. The roots and tops of the plants grown in the less concentrated solutions were high in proportion of young active cells containing dense protoplasm. All tissues differentiated and matured slowly. Carbohydrates were relatively low and organic nitrogen high. A large proportion of the elaborated nitrogen was in the form of amide and amino nitrogen. Nitrate was present in abundance.

4. All organs of the cultures supplied with the more concentrated solutions were relatively low in proportion of young cells with dense protoplasmic contents. Tissues differentiated and matured rapidly. There were present strongly developed fibers and other mechanical elements. Carbohydrates were high and organic nitrogen relatively low. A large proportion of the elaborated nitrogen was in the form of complex proteins. Nitrate was present in abundance.

5. With a deficiency of carbohydrates and slow maturation of tissues of the low concentration cultures, there persisted at the junction point of pedicel and peduncle several tiers of cells which failed to mature and remained meristematic in appearance. These tiers of cells were approximately at right angles to the main axis of the pedicel. Ultimately the middle lamellae of one or more tiers became gelatinized, accompanying which the cells separated and the flower bud abscissed. This mode of derivation of the abscission layer is in contrast to the abscission through the non-succulent pedicels of mature fruits, such as the apple, or abscission through the comparatively woody petioles of mature leaves, in which case mature cells apparently become active and thus give rise to the tiers of meristematic cells (29) forming the abscission layer.

6. It is a well recognized fact that the amount of water absorbed from a concentrated solution of salts is much less than from a more dilute solution (16). As already recorded, the plants of the cultures

supplied with concentrated nutrient were lacking in succulence and otherwise xerophytic in character.

7. Although the percentage of nitrate was fairly high in the plants of all the nutrient series, an effect of a high concentration of nutrient salts was practically that of low nitrogen nutrition, or more correctly, low protein nutrition.

8. Only comparatively young cells containing abundant protoplasm were capable of synthesis of proteins from nitrate.

9. As the plants of the high concentration cultures had relatively few young active cells, they exhibited only limited reduction and assimilation of nitrate and there was accordingly much less oxidation of carbohydrates or their derivatives. This fact would seem to account, at least in part, for the marked accumulation of carbohydrates.

10. Relationships of soil moisture content and salt concentrations in the soil are discussed and it is pointed out that, long before the wilting point is reached, the salt concentration of the soil solution may bring about early maturation of tissues.

11. Mature, optically empty tissues being low or entirely lacking in ability to synthesize proteins from nitrate, there is brought about a condition of protein deficiency incorrectly called nitrogen deficiency, since under the conditions mentioned, nitrate may be present in abundance in the nutrient medium and in the plants. The fact is also emphasized that such effects are especially severe in case of fruit trees, some grasses, and other perennials, because such plants carry on the initial phases of nitrate assimilation and protein synthesis almost exclusively in the fine succulent rootlets. These organs mature and lose ability to manufacture protein very rapidly when exposed to a relatively concentrated solution of salts. The roots, however, can absorb nitrate and other nutrients freely if they are present in the solution of the nutrient medium.

EXPERIMENT STATION
PINEAPPLE PRODUCERS COOPERATIVE ASSOCIATION
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LITERATURE CITED

1. BLAKE, M. A., and DAVIDSON, O. W., The New Jersey standard for judging the growth status of the Delicious apple. New Jersey Agr. Exp. Sta. Bull. 559. 1934.
2. CONRAD, J. P., and VEIHMEYER, F. G., Root development and soil moisture. Hilgardia 4:113–134. 1929.
3. DAVIDSON, O. W., and SHIVE, J. W., The influence of the hydrogen-ion concentration of the culture solution upon the absorption and assimilation of nitrate and ammonium nitrogen by peach trees grown in sand cultures. Soil Sci. 37:357–382. 1934.
4. ———, Determination of the nitrogenous fractions in vegetative tissue of the peach. Plant Physiol. 10:73–92. 1935.
5. ECKERSON, SOPHIA H., Microchemical studies in the progressive development of the wheat plant. Washington Agr. Exp. Sta. Bull. 139. 1917.
6. ———, Protein synthesis by plants. I. Nitrate reduction. BOT. GAZ. 77:377–390. 1924.
7. ———, Influence of phosphorus deficiency on metabolism of the tomato. Contrib. Boyce Thompson Inst. 3:197–217. 1931.
8. ———, Seasonal distribution of reducase in the various organs of an apple tree. Contrib. Boyce Thompson Inst. 3:405–412. 1931.
9. ———, Conditions affecting nitrate reduction by plants. Contrib. Boyce Thompson Inst. 4:119–130. 1932.
10. ———, Microchemistry (mimeographed).
11. EMMERT, E. M., and BALL, F. K., The effect of soil moisture on the availability of nitrate, phosphate, and potassium to the tomato plant. Soil Sci. 35:295–306. 1933.
12. FAGAN, T. W., The variation in the moisture and nitrogen content of the potato during growth and storage. Welsh Jour. Agr. 1:110–118. 1925.
13. GOURLEY, J. H., Anatomy of the transition region of *Pisum sativum*. BOT. GAZ. 92:367–383. 1931.
14. HODGSON, R. W., An account of the mode of foliar abscission in *Citrus*. Univ. California Publ. in Botany 6:417–428. 1918.
15. KRAUS, E. J., and KRAYBILL, H. R., Vegetation and reproduction with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149. 1918.
16. MAXIMOV, N. A., The plant in relation to water. Allen and Unwin, London. 1929.
17. MOTHES, K., Die natürliche regulation des pflanzlichen eiweisstoffwechsels. Ber. Deutsch. Bot. Ges. 51:31–46. 1933.
18. NIGHTINGALE, G. T., Effects of temperature on metabolism in tomato. BOT. GAZ. 95:35–58. 1933.
19. ———, Ammonium and nitrate nutrition of dormant Delicious apple trees at 48° F. BOT. GAZ. 95:437–452. 1934.

20. ———, Effects of temperature on the growth, anatomy, and metabolism of apple and peach roots. Bot. Gaz. 96:581–639. 1935.

21. NIGHTINGALE, G. T., ADDOMS, RUTH M., ROBBINS, W. R , and SCHERMER-HORN, L. G., Effects of calcium deficiency on nitrate absorption and on metabolism in tomato. Plant Physiol. 6:605–630. 1931.

22. NIGHTINGALE, G. T., and BLAKE, M. A., Effects of temperature on the growth and composition of Stayman and Baldwin apple trees. New Jersey Agr. Exp. Sta. Bull. 566. 1934.

23. ———, Effects of temperature on the growth and metabolism of Elberta peach trees with notes on the growth responses of other varieties. New Jersey Agr. Exp. Sta. Bull. 567. 1934.

24. NIGHTINGALE, G. T., and MITCHELL, J. W., Effects of humidity on metabolism in tomato and apple. Plant Physiol. 9:217–236. 1934.

25. NIGHTINGALE, G. T., and ROBBINS, W. R., Some phases of nitrogen metabolism in *Polyanthus narcissus*. New Jersey Agr. Exp. Sta. Bull. 472. 1928.

26. NIGHTINGALE, G. T., and SCHERMERHORN, L. G., Nitrate assimilation by asparagus in the absence of light. New Jersey Agr. Exp. Sta. Bull. 476. 1928.

27. NIGHTINGALE, G. T., SCHERMERHORN, L. G., and ROBBINS, W. R., The growth status of the tomato as correlated with organic nitrogen and carbohydrates in roots, stems, and leaves. New Jersey Agr. Exp. Sta. Bull. 461. 1928.

28. PEARSALL, W. H., and EWING, J., The relation of nitrogen metabolism to plant succulence. Ann. Bot. 43:27–34. 1929.

29. SAMPSON, H. C., Chemical changes accompanying abscission in *Coleus blumei*. Bot. Gaz. 66:32–53. 1918.

30. SANI, G., Intorno all'attivita riduttrice delle graminacee; la riduzione del nitrato di calcio per le radici delle graminacee. Atti. Accad. Lincei. 10:197–201. 1929.

31. SHIVE, J. W., and STAHL, A. L., Constant rates of continuous solution renewal for plants in water cultures. Bot. Gaz. 84:317–323. 1927.

32. TIEDJENS, V. A., Factors affecting assimilation of ammonium and nitrate nitrogen particularly in tomato and in apple. Plant Physiol. 9:31–57. 1934.

33. VEIHMEYER, F. J., Some factors affecting irrigation requirements of deciduous orchards. Hilgardia 2:125–284. 1927.

34. WELTON, F. A., Lodging in oats and wheat. Bot. Gaz. 85:121–151. 1928.

35. WERNER, H. O., The effect of controlled nitrogen supply with different temperatures and photoperiods upon the development of the potato plant. Nebraska Agr. Exp. Sta. Res. Bull. 75. 1934.

RATIO OF WATER CONTENT TO DRY WEIGHT IN LEAVES OF THE CREOSOTE BUSH

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(WITH NINE FIGURES)

Introduction

The general characteristics of the remarkably drought resistant creosote bush, *Larrea tridentata* Cav., have recently been discussed and the structure and life history of the leaves portrayed (23). Part of the foliage is persistent through long drought. The same leaf may actually have two periods of growth separated by a season of drought. In the work here reported it was found that this drought resistant foliage is characterized by an exceptionally low water content and high saturation deficit. Other foliage on the same branches is unable to withstand a decrease in moisture content much below a level characteristic of many mesophytic woody plants. By a simple method it is shown that leaves with the same moisture content may differ markedly in their access to a water supply. The investigations were made in the vicinity of the Desert Laboratory of the Carnegie Institution of Washington, Tucson, Arizona, during the summers of 1928–31.

Literature

Apparently no special study of the water content of *Larrea* has previously been made. LIVINGSTON and BROWN (15), the first to publish data in this country concerning water content changes in leaves, give four measurements for *Larrea* foliage (showing a change not confirmed by the present work). SCHRATZ (25), in a study of various aspects of thé water physiology of Arizona desert plants, includes 12 moisture determinations on leaves of *Larrea* for the purpose of indicating the influence of time of day and of habitat. ASHBY (1) has added three more determinations to this short list. The data presented in these papers indicate that the foliage has a low and fluctuating water content, which may be from about one-half to two times the dry weight.

Prominent in the general literature pertaining to the water balance of plant tissues are the publications of YAPP and MASON (41), WALTER (40), MAXIMOV (18), PISEK and CARTELLIERI (22), and STOCKER (31–35). Earlier literature is reviewed by these authors. While many of the methods employed in these studies are open to certain criticisms, the facts in regard to water content stand out prominently. Each species has a somewhat characteristic water content which changes within limits which are also rather well defined. In some species, especially those of dry habitat, changes are very marked; in others, especially shade mesophytes, the water content remains well poised. In any habitat, however, there are likely to be both types of plants, some physiologically capable and some incapable of withstanding wide variations of water content. Inasmuch as fluctuations are due to a varying balance between water intake and loss, they form a valuable index to the water conditions of the plant.

True water content change, however, is rarely measured. Change of *percentage* water content is another quantity. Most often, percentage water is interpreted simply as absolute water content. This may result in magnification or in masking of the true changes of water content. Thus, for example, differences in actual water content are probably less than differences in some of the percentage data of KRASNOSSELSKY-MAXIMOV (19) and of SCHRATZ (25), because of changes of dry weight in direction opposite to those of water. YAPP and MASON (41) even draw conclusions as to turgor differences from their percentage data. The experimental conditions in some of YAPP and MASON's experiments were certainly such as to induce marked dry weight changes, exaggerating water content differences. MAXIMOV and KRASNOSSELSKY-MAXIMOV (19) have shown in one case how the rapid disappearance of organic reserves in wilting leaves may prevent their showing a percentage water content below that of turgid controls.

Several investigators, using plants of high moisture content (5 to 10 times the dry weight), have expressed their data as percentage of the *fresh weight*. When this is done, extensive changes in actual water content make comparatively small changes in percentage water content, as may be seen in figure 1. The water percentage of the fresh

weight changes progressively less and less as the absolute water content increases if the dry weight is constant. Water as percentage of the dry weight, on the other hand, is a linear relation of the absolute water content (fig. 1). The masking effect of the fresh weight basis of computation makes it seem possible that the actual water content changes in the plants investigated by KNIGHT (12) and by GILBERT and ADAMS (8) may have been greater than is indicated by their data.
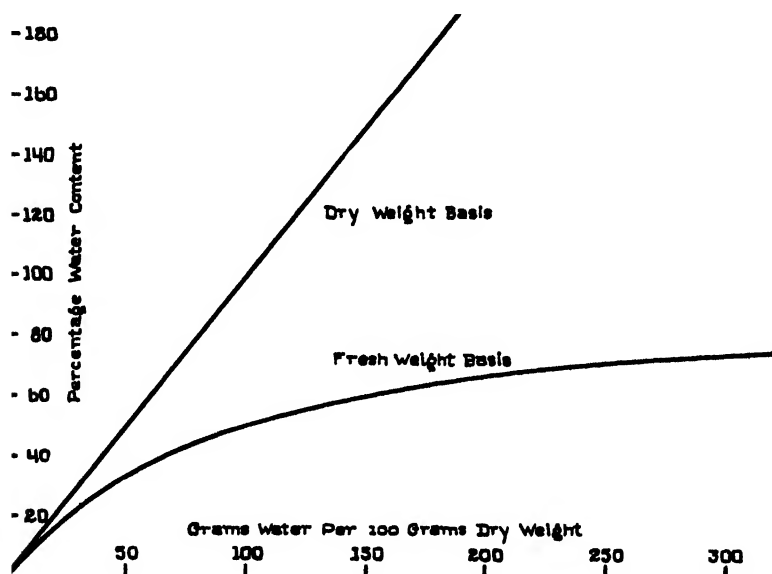
FIG 1.—Relation between water content and percentage water content. Comparison of dry and fresh weight bases of computation.

The moisture content does change even in shade plants. Percentage water content data are supported by data obtained by other methods, as by volume changes (2, 36) and the difference between absorption and transpiration (29). DENNY (7) has followed diurnal changes in leaves by methods which permit approximate determination of absolute changes. He has demonstrated fluctuations in absolute water content so great as to show that the fresh weight basis of comparison is unsuitable for most of the varieties (about 20) he used. The variations in absolute water content might have been greater had he collected his material before 4 P.M., when the mini-

mum percentage water content frequently occurs. In the woody plants DENNY used, the dry weight did not change appreciably during the night. The numerous correlations which have definitely been established between percentage water content changes and changes in the water conditions are further evidence that true water content changes are responsible for much of the observed percentage changes.

On the other hand, water content is affected not alone by water conditions. Osmotic, hydrational, and wall pressures, which influence the water content of a cell, may be markedly affected by the nature and concentration of the ions present. Water content will therefore vary with the nutrient conditions of the habitat (14). Differences in the water conductivity of stems may be expected to influence the water content of leaf cells (28). Flowering or tuber formation may possibly influence the amount of foliar moisture (4, 3, 10). Finally, light undoubtedly influences the capacity of cells to hold water. Interpretation of the meaning of water content data must therefore be made cautiously. Nevertheless, especially in desert regions where all vegetation is dependent on its water conditions, nutrient and other differences (although of unquestionable influence) are probably of minor importance as compared with water supply in affecting water content.

A small number of water content measurements on any one species can have little significance because of the likelihood of sampling errors and because meaning of the data procured is obscure without bases of comparison, that is, standard water contents associated with some definite conditions or activities of the plant. For interpretation of the existing water content, several investigators have compared it with the value attained when leaf cells are brought to approximate saturation with water (37, 11, 26, 4, 31–35, 21, 22). For attainment of saturation, shoots are usually put in moist chambers, their cut stems in water, for 12 to 72 or more hours. VASSILJEV (39) objects to this method in that it leads to values which are too high; periods as long as three days in a moist chamber probably do lead to erroneous results. However, the accumulated data give some idea of the degree to which the cell walls normally are distended. Some plants are always far below saturation; others suffer injury if their water content is reduced much below their maximum water holding

capacity. The difference between the percentage water content at saturation and the existing percentage water content has usually been spoken of as the saturation, or water deficit.

OPPENHEIMER (21) recommends determination of sublethal water content, which is the percentage of the saturation water content which may be lost before death of detached leaves or shoots left on a laboratory table perhaps two or three weeks. The likelihood of distinctly pathological effects would seem to restrict the utility of this method. Where measurable, a much more reliable and significant water content is that at permanent wilting (5, 13, 17). Even this minimum water content may be rather indefinite (20), and is not determinable for a plant like *Larrea*, which never wilts and normally shows large changes in moisture content.

Knowledge of the degree of hydration of the chloroplasts, cytoplasm, nucleus, or other portions of the protoplast would be more significant than knowledge of the total "free" water content of tissues. However, as yet we have no method for determining the water content of even the protoplasm alone as distinct from the associated non-living portions. The finest methods are gross in comparison with the intricacy of organization of the living plant.

Methods

Leaves are picked from the bush and put into tared weighing bottles or vials, which are then kept tightly stoppered and as cool as possible. The extreme importance of careful sampling will be evident from the data presented. The inclusion of parts other than leaves (twigs, galls, flowers, or fruits) may introduce considerable error. If weighing cannot be made immediately after picking the leaves, the bottles are inclosed in a humid box. Weighings are made to milligrams. More accurate weighing is unnecessary (except for very small samples) in view of the magnitude of variations inevitable in sampling. After weighing the leaves, the stoppers are removed and the leaves heated in an electric oven for about 24 hours. In most cases leaf samples are completely dry in less than 12 hours, but as one day is a more convenient interval, and since no appreciable change in weight of *Larrea* leaves occurs even with much longer heating, the 1 day period has been adopted. At no time in Tucson

has it been found necessary to use a desiccator; the dry leaf material is not sufficiently hygroscopic to increase significantly in weight even after standing open to the air for 20 minutes. Weighings, however, are always made immediately after the stoppered containers have sufficiently cooled.

To emphasize the possibility of a varying dry weight, data are given for the most part as the ratio of water content to dry weight (wc:dw) instead of in percentage figures. Percentage water content is of course 100 times the value of the ratio. Wc:dw ratios at different times of day, at different seasons, at water saturation, and when transpiration is eliminated are compared. The special methods required for the determination of these ratios are described in their respective sections.

I. THE WC:DW RATIO UNDER NATURAL CONDITIONS

LEAVES OF DIFFERENT AGE.—The creosote bush has an abundance of fine branches. One side branch is usually to be found at each node. Under favorable conditions, that is, ample soil water, every branch is densely clothed with tiny leaves, two at a node for seven to ten nodes from the apex. The wc:dw ratio of leaves of different age as found contemporaneously at these different nodes is markedly different. The sampling method used to determine this was to select several (6–20) leafy shoots, and to put all the buds in one weighing bottle and all of the leaves at each successively lower node into separate weighing bottles. In some cases all of the leaves were divided into only two or three groups, each group containing the leaves of two or more nodes from each of the several twigs. All of the data obtained fall into either one or the other of two contrary groups, whose characteristics are summarized in tabulation shown on page 524. Figure 2 shows graphically data selected from the two groups. These two seemingly irreconcilable situations will be discussed later.

Clearly, since so great differences in percentage water content are to be found even at adjacent nodes of *Larrea* stems, uniform successive samples from the same bush can be obtained only by careful selection of leaves of exactly the same age at each picking.

LEAVES FROM DIFFERENT BRANCHES OF SAME BUSH.—The leaf wc:dw ratio of one branch may or may not be closely similar to that

of other branches. Differences as great as 20 per cent may exist between different branches on the same bush. Even when branches are selected for close uniformity, as when two forks ("twins") of a Y-shaped branch are separately sampled, differences as great as 5 per cent are sometimes shown. By very careful selection closer replication is usually obtainable, but in following progressive changes of the wc:dw ratio, differences less than 5 per cent are of doubtful significance.

DATA OF GROUP I	DATA OF GROUP II
—show the wc:dw ratio to be higher at each successively lower node; that is, the ratio increases with age.	—show the wc:dw ratio to be lower at each successively lower node; that is, the ratio decreases with age.
—show a rather consistent uniformly ascending gradient from one node to the next below.	—show not so consistently a uniformly descending gradient from one node to the next below.
—comprise all the determinations made on dates other than Aug. 9–12, 1931, namely, 9 dates between June 15 and Sept. 2, including periods of drought dormancy as well as periods of rapid growth.	—comprise all the determinations made on the four consecutive days Aug. 9–12, 1931, and none other,[1] a period of very rapid growth.
—show wc:dw ratios of 0.63–1.68.	—show wc:dw ratios of 1.20–1.98, the latter being the highest recorded for *Larrea* in the present investigations.
—comprise 63 determinations, involved in 18 tests, made on 12 bushes.	—comprise 60 determinations, involved in 14 tests, made on 7 bushes.

[1] In two of the three tests at the University of Cincinnati, greenhouse-grown *Larrea* showed the maximum wc:dw ratio in the buds. The water content of the buds and youngest leaves was more than twice the dry weight.

VARIATIONS WITH SEASON AND HABITAT.—The purpose of these investigations was to determine the nature of the existing variations of the wc:dw ratio. Except in a general way, no attempt was made to correlate these variations with environmental factors.

Adjacent bushes in an apparently uniform habitat frequently deviate conspicuously in water content, indicating probable local differences in water conditions. Thus for example, within an area
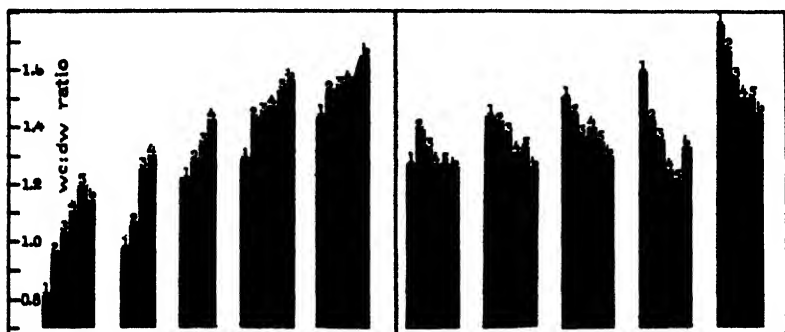
FIG. 2.—The wc:dw ratio of leaves at different nodes. Each diagram represents the relative wc:dw ratio of leaves at successive nodes (1, 2, 3, 4, 5, 6) from apex. The five diagrams at the left represent the usual situation, the oldest leaves having the highest wc:dw ratio; the five diagrams at the right show the reverse situation, which occurred during a period of rapid growth.

of about an acre on Tumamoc Hill six individuals were found to have wc:dw ratios of from 0.71 to 1.13; in another locality six bushes ranged in wc:dw from 0.77 to 0.97. In these tests leaf samples were taken from all parts of the selected bushes, as nearly simultaneously as possible, sometimes with the aid of an assistant. The differences shown are for the most part unpredictable from external appearances, although brighter green foliage frequently has higher percentage water content.

The wc:dw ratios for leaves of bushes in habitats which are obviously different are correspondingly different. In July, 1931, on Tumamoc Hill there had been a number of rains and the ratio was greater than 1.0 (1.10–1.33). At the same time, 8 miles to the south (Ajo Pass, Tucson Mountains) where no considerable rain had fallen, the ratio was little more than half this amount (0.54–0.67). Similar variations occur in the same bush before and after the summer rains.

Determinations made in late June and early July, 1928, on bushes in the vicinity of the Desert Laboratory, showed their average ratio to be 0.54. Following the midsummer rains, the average was 1.35. These data represent 77 tests on 18 bushes. In table I are other specimen ratios of individual bushes before and after periods of rain or watering. Between one growing season and the next, weeks or even months of excessively dry hot weather may intervene, while the percentage water content of all leaves retained on the twigs drops

TABLE I

SEASONAL VARIATION OF WC:DW RATIO

BUSH	BEFORE RAIN PERIOD		PRECIPI-TATION (CM.)	AFTER RAIN PERIOD		PERCENT-AGE IN-CREASE
	DATE	WC:DW		WC:DW	DATE	
L.........	7/26/28	0.59	8 7	1.15	8/15/28	49
8.........	7/31/28	0.59	4.9	1 12	8/ 9/28	47
Bj........	7/15/31	0.60	10 4	1 30	8/27/31	54
P.........	7/26/28	0 71	6 6	1.30	8/ 9/28	45
b*........	7/31/30	0 74	*	1.25	9/26/30	41
y*........	7/27/30	0 89	*	1 95	8/14/30	54
a*.......	7/ 4/30	1 04	*	1 56	7/31/30	33

* Artificially watered.

sharply. If the dry period is long and severe, the water content may fall to less than one-half the dry weight.

From the data on nodal and seasonal differences, we may conclude that a leaf ordinarily undergoes an increase in percentage water content as it comes to maturity, and then a decrease due to seasonal drought.

DIURNAL VARIATIONS.—Several hundred ratio measurements were made during the summers of 1928, 1930, and 1931 in order to obtain a complete picture of the changes that take place from hour to hour of the day and night. Since the simultaneously existing differences from node to node or from branch to branch are often greater than in the same leaves from hour to hour, sampling must be done with the greatest care. One satisfactory procedure is to select several shoots which by previous test have proved to have nearly identical leaf wc:dw ratios, and which have the same exposure, density of

foliage, etc.; to use then the leaves of one shoot for each time of day a sample is desired.
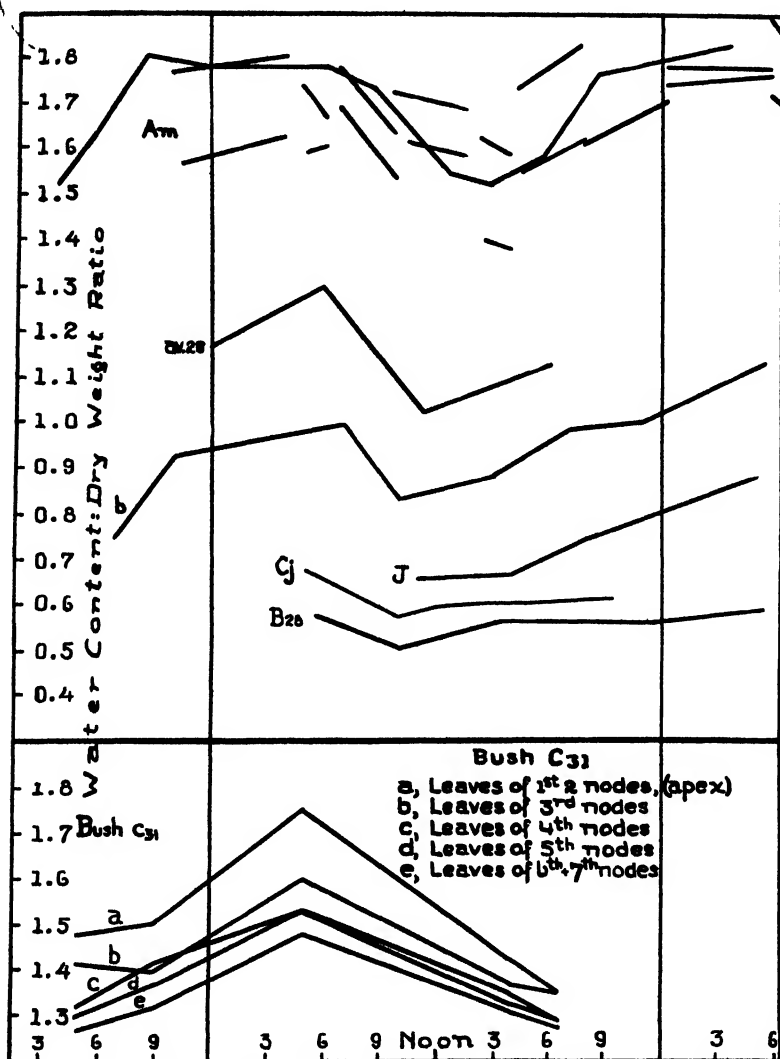
FIG. 3.—Diurnal variation of wc:dw ratio of leaves of different bushes

Graphs representative of the most carefully executed experiments are shown in figure 3. Many other graphs obtained follow the same

curves; a few are of different form, or show erratic fluctuations. The latter results are probably due partly to faulty sampling, and partly to obscure circumstances which derange the normal changes. The curves for bush Am in figure 3 consist of (1) a continuous line curve each point of which represents the water-dry weight ratio of the leaves of a single twig, all of the twigs having been chosen for strict uniformity; and (2) short lines, each of which joins the wc:dw ratio of half of the leaves of a selected shoot at one time and the wc:dw ratio of the remaining leaves 2–6 hours later. The short lines rarely coincide in position with that of the continuous line curve, indicating again that the level of percentage water content may be markedly different in various parts of a bush. It is significant that the same general direction of change appears consistently in all the curves for bush Am. The changing ratio in leaves *at different nodes* is shown in the curves for bush C31. Here all the shoots used had been shown to be uniform in wc:dw ratio. It will be seen that these readings were taken when all bushes tested had a lower ratio in the lower leaves. The other curves of figure 3 represent the average condition of leaves of all ages, and taken from all parts of the bushes indicated. It is clear that inasmuch as these do not show the variations at different nodes and branches, they represent an incomplete picture. A composite of curves must be given to express diurnal variation adequately.

The wc:dw ratio is greatest at daybreak. A sharp decline in the ratio begins at sunrise, leading to a minimum value which may occur at any time between 10 A.M. and 7 P.M. If the minimum occurs early in the day, there is a gradual increase during the afternoon or a low fluctuating ratio until sunset when there starts a steady increase. The difference between the maximum and minimum values for any 24 hour period is from about 0.03 to 0.60, or 10 to 35 per cent of the maximum value.

SEPARATE RÔLES OF WATER CONTENT AND OF DRY WEIGHT IN CHANGES OF THE WC:DW RATIO.—It is to be expected that dry weight changes, if any, are in a direction opposite to those of water content. This follows from a consideration of the usual periodicity of transpiration and photosynthesis. Some evidence in regard to the occurrence of dry weight changes in *Larrea* is afforded by I₂KI-starch

tests made on leaves picked at different times of day. It is found that leaves of irrigated bushes contain conspicuously more starch at 5 P.M. than at 5 A.M. Leaves of bushes not irrigated contain very little starch, however, and exhibit a very inconspicuous difference in the starch content at 5 P.M. and 5 A.M. A change in starch content does not necessarily mean a change in dry weight. But there is a suggestion here that leaf dry weight changes may be more pronounced in bushes amply supplied with water.

A number of attempts were made in the field to determine how much change takes place in the absolute dry weight and water content from hour to hour. In order to do this, one leaf of the pair at each of several nodes was picked at one time, and the other leaf at the same nodes, closely matched as to size and exposure, picked at the second time. Obviously success in such work, especially with such small leaves, requires the utmost care to insure exact replication. Uncontrollable environmental conditions and limited time prevented satisfactory results in Tucson, in lieu of which similar experiments were undertaken at the University of Cincinnati under more favorable, but artificial, conditions, and with very limited material. The plants used were in pots placed out of doors and fully exposed to the sun and wind. The results are given in tables II and III.

As seen in the tables, the two leaves at the same node are designated *a* and *b*. In further experiments, the half-leaf method was used, the two pinnae of the same leaf being separately tested and designated *a* and *b* respectively. Table II shows how closely similar *a* and *b* are when sampled simultaneously. Table III gives the values at about the time the ratio is at maximum and at minimum. Although the data in the tables are meager, some facts seem clear:

1. Except in experiment 1 (in which only three leaves were included in a sample) and in experiment 3, the two leaves at the same node or the two halves of the same leaf as sampled simultaneously differ from each other less than 3 per cent.

2. In experiment 3, the higher difference (4.4 per cent) in the dry weight and milligrams of water does not appear in the percentage water content, making it seem very probable that equivalent areas were not included in *a* and *b;* that is, that there was an error in sampling.

3. The differences shown in table III are greater than those in table II. They are, moreover, in the expected directions. The data are therefore sufficient to demonstrate a diurnal change in dry weight, as well as in water under the conditions of the experiments.

4. Except in experiment 6, the change in water content is greater than the change in dry weight.

TABLE II

COMPARISON OF THE TWO LEAVES (A AND B) AT SAME NODE AND OF THE
TWO HALVES (A AND B) OF SAME LEAF (UNIVERSITY OF CINCINNATI)

EXPERIMENT NO AND METHOD	DRY WFIGHT			WATER CONTENT			WATER CONTENT (% DRY WEIGHT)		
	A (MG)	B (MG)	PER-CENT-AGE DIF-FER-ENCE*	A (MG)	B (MG)	PER-CENT-AGE DIF FER-ENCE*	A (%)	B (%)	PER-CENT-AGF DIF-FER-FNCE*
Opposite leaves (a and b)									
1. Leaves of 3 nodes	5 8	5 6	3 50	12 1	12 7	4 84	209	227	8 25
2 Leaves of 6 nodes	8 8	8 9	1 13	19 1	19 0	0 52	217	214	1 39
Average per leaf (9 nodes)	1 622	1 611	0 68	3 467	3 522	1 56	214	218	1 85
Half leaves (a and b)									
3 10 half leaves	11 5	11 0	4 44	20 8	19 9	4 41	181	181	0
4 10 half leaves	13 1	13 2	0 76	26 3	26 8	1 88	201	203	0 99
5. 15 half leaves	17 3	16 8	2 93	32 1	31 7	1 88	187	189	1 06

* In percentage of $\frac{a+b}{2}$.

5. Water content and dry weight changes are in opposite directions as predicted. The wc:dw ratio therefore changes more than does the actual water content.

Further information regarding the diurnal changes in *Larrea* leaves is afforded by study of the osmotic concentration of the cell sap.

DIURNAL CHANGE OF OSMOTIC CONCENTRATION AND WC:DW RATIO.—Dr. T. D. MALLERY and the writer conducted an experiment to determine the nature of the diurnal fluctuation of osmotic and wc:dw ratio values (16). Two experimental bushes, Am and Bm, were selected as representing contrasting foliage characteristics.

Recent rains had provided enough soil water to permit growth, but Bm appeared less favorably situated than Am. The leaf size and rate of growth were evidently greater for bush Am. (WALTER's (40) figure 26, p. 65, shows Am). At intervals of 2–4 hours for 50 hours, leaf-plus-twig samples were taken from all over the bushes for osmotic and water content data. Simultaneously, leaf samples from care-

TABLE III

DIURNAL CHANGE OF DRY WEIGHT, WATER CONTENT, AND PERCENTAGE WATER CONTENT (UNIVERSITY OF CINCINNATI)

EXPERIMENT NO., METHOD, AND TIME INTERVAL	DRY WEIGHT (MG)			WATER CONTENT (MG)			WATER (% OF DRY WEIGHT)			WATER (% OF FRESH WEIGHT)		
	A	B	CHANGE*	A	B	CHANGE*	A	B	CHANGE*	A	B	CHANGE*
Opposite leaf method 6 9 pairs of leaves 5 00 P M (=a) 4 30 A M (=b)	23 6	21 7	−8 4	37 9	39 1	+3 1	160 0	180 0	+11 8	59 5	64 8	+8 5
Half leaf method 7 10 leaves 5 00 P M (=a) 4 30 A M (=b)	9 5	9 0	−5 4	14 0	16 6	+16 3	147 0	184 0	+22 2	61 6	64 3	+4 3
8 10 leaves 5 00 P M (=a) 4 30 P M (=b)	11 9	11 4	−4 3	18 8	21 3	+12 2	158	187 0	+16 4	61 2	65 1	+6 2
Average (6+7+8) 5 P M −4 30 A M	15 0	14 0	−6 5	23 6	25 7	+8 2	155 0	184 0	+17 0	60 8	64 7	+6 2
9 13 leaves 6 00 A M (=a) 3 30 P M (=b)	16 3	17 0	+4 2	31 6	27 4	−14 2	194 0	161 0	−19 0	06 0	61 7	−6 7

* In percentage of $\dfrac{a+b}{2}$

fully selected individual branches were taken for wc:dw ratio determination. Some of the results are plotted in figure 4 (cf. MALLERY's fig. 5).

Evidence in regard to diurnal change of dry weight lies in the constancy of the product. osmotic value times wc:dw ratio (22). The product may be designated thus:

$$\frac{kc}{Ws}\left(\frac{Ws+Wr}{c+d}\right),$$

where c = number of millimols of osmotic species, (c+d) = grams dry
weight, Ws = grams of solvent water, (Ws+Wr) = grams total water
content, and k = a constant. If the solvent water and the total water
content are almost equal in the leaf tissue, that is, if Ws and (Ws+
Wr) in the product balance each other, then it follows that the
product is not much affected by changing water content. On the
other hand, total dry weight is undoubtedly considerably greater
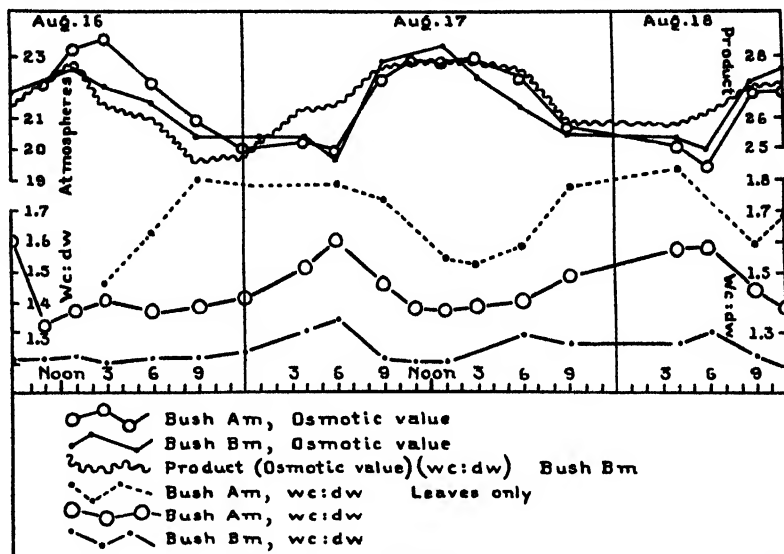
FIG. 4.—Diurnal changes in foliage (leaves plus green twigs) of two bushes, Am
and Bm.

than the osmotic portion of it, so that if the product varies consider-
ably, the dry weight is changing (as well as, probably, the water
content).

In the present experiment the product varied quite as much as did
the factors of the product (fig. 4). According to the foregoing, this
means that in these bushes diurnal changes of the dry weight were oc-
curring. Since the validity of the assumptions involved in this inter-
pretation is open to some question, the data must be considered not
as proof but merely as evidence additional to that presented in the
previous section, pointing to the same conclusion, that diurnal dry
weight fluctuations do occur in *Larrea* bushes well supplied with
water.

The course of the diurnal changes need not be discussed further than (1) to call attention to the inverse direction of change of osmotic and ratio values, (2) to note that the interpretation (as by WALTER 40) of such a relationship to mean that water content changes are the cause of the osmotic changes is unjustified in view of the probable dry weight fluctuations, and (3) to note that the changes shown are in the directions which would be expected in view of the periodicity of photosynthesis and transpiration.
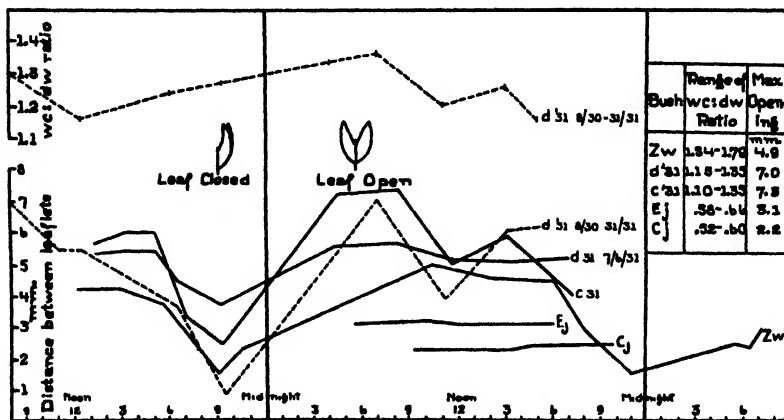
FIG. 5 —Diurnal movement of *Larrea* leaflets and representative curve of the march of percentage water content No relation exists between time of leaf opening or closing and level of percentage water content.

LEAFLET MOVEMENT; RELATION TO DIURNAL CHANGE IN WC:DW RATIO.—*Larrea* exhibits nyctinastic leaflet movements. In the hope of finding a correlation between the march of diurnal water content variations and the movements, as reported by TRELEASE (38) for palm leaves, exact measurements of the positions of *Larrea* leaflets were made at various times of day and night, and concurrently, wc:dw ratio determinations on similar leaflets. No correspondence appeared, however. The time of maximum opening (7–11 A.M.) occurs two or more hours after the time the water content is at its maximum (sunrise); and the time of minimum opening (7–11 P.M.) occurs many hours after the time of day water content is at a minimum (fig. 5). Evidently the changes in water content responsible for the movements are localized in the motor tissue, and are not general throughout the leaf.

General discussion.—*Larrea* foliage exhibits some striking characteristics. Most outstanding are the changes that may take place in the life cycle of a single leaf, and the lowness of the water content. Analysis of the wc:dw data here reported clarifies and amplifies the concepts previously presented (23) showing *Larrea* to have a peculiar admixture of xeric and mesic characteristics.

During the growing season the leaves have a water content level almost identical with that of leaves of such genera as *Quercus*, *Castanea*, *Hamamelis*, and *Fagus* (27, 6): between one and two times the dry weight. Indeed most of the foliage at this season in water content as well as in structure gives no evidence of the xeric nature of *Larrea*. Drought resistance is characteristic of only a small proportion of the leaves.

It is possible to construct diagrams showing roughly the wc:dw ratios for representative pairs of leaves that develop successively on a twig, and the change in the ratios from one season to the next during the summer (fig. 6). This is done by correlation of the nodal and seasonal wc:dw ratio data with the life duration and growth studies (23), in which it was established that seasonal changes represent not merely a substitution of new leaves for old ones, but an actual change in the same leaves from one season to the next. For convenience, and according to their time of development, three types of leaves, *a*, *b*, and *c*, may be distinguished.

The *a* type of leaf begins its development early in a growing season and is thus subject during its whole developmental period to relatively mesic conditions. Loss of much water is fatal to leaves of the *a* type, and they therefore fall early in the dry period or early in the next growing season. The data on nodal variation show that younger leaves regularly have a lower water content than older ones. The leaves designated *b* and *c* are therefore represented in figure 6 with narrower bands than' the *a* leaves, which have previously expanded from the bud. Examples of the *b* type of leaves occur infrequently. They do not attain full size before the dry season checks further growth. They survive the drought and show increased water content but no further growth during the second growing season. It is the *c* leaves which have two periods of growth. These leaves begin their development during a period of increasing drought, last through the dry season with the lowest water content of any of the

foliage, and on the return of rain, not only increase their water content but resume growth. They are shed in the subsequent dry period. The three leaf types listed in the order *a*, *b*, and *c* are arranged according to increasing life duration, increasing drought resistance, and decreasing water content. Of all the leaves, the *a1* subtype (fig. 6) is most abundant. They have a comparatively high wc:dw ratio
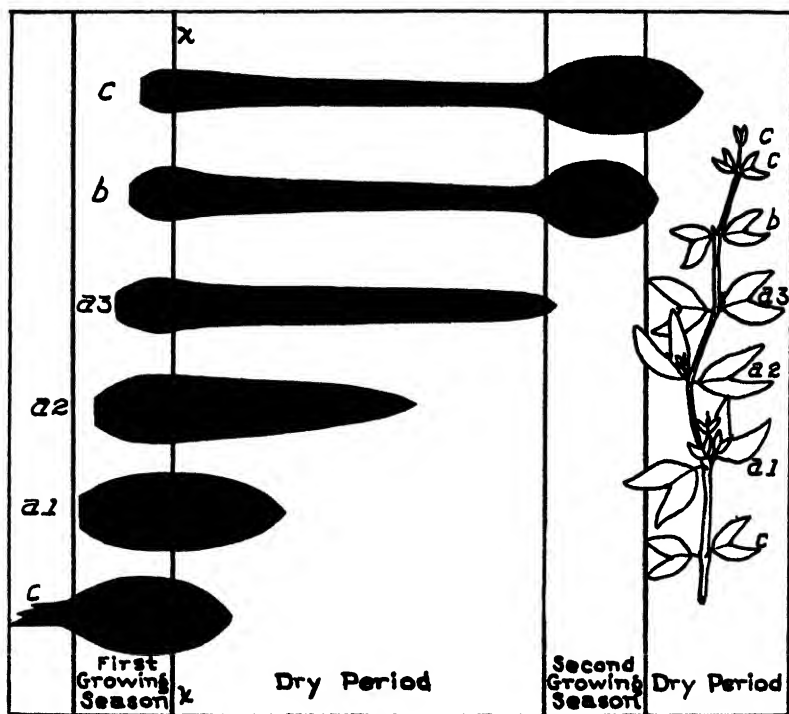
FIG. 6.—Schematic diagram representing life span and percentage water content of *Larrea* leaves. Relative water content indicated by thickness of bands: where the bands become narrower the water content is decreasing, and vice versa. The twig shows the relative positions of different leaf types at end of a growing season (at time indicated by *x* on left side of figure).

throughout their brief lives and are not drought resistant. The drought resistant *c* leaves look exactly the same except for their small size.[2]

[2] Figure 6 simplifies the true picture of percentage water content changes in the life of a leaf. Diurnal variations are not shown; the summer dry seasons are often broken by

It is to the *b* and *c* leaves that *Larrea* owes its reputation of being the most extreme xerophyte of the southern deserts of North America. The *c* leaves during a severe drought have a water content which is lower (except for air-dry plants or plant parts) than any other found reported in the literature, less than 50 per cent.[3]

Any *a* leaves (a_2, a_3) which are retained during a portion of the drought have also a very low water content, not very much more than the *c* leaves adjacent to them at the stem apex. Tremendously different in physiological capacity, these two leaf types are nevertheless quite similar in appearance, structure, and even in water content. In this respect *Larrea* differs from one of its companion species, *Encelia farinosa* (26).

A study of the degree of vacuolization and the chemical characteristics of the protoplasm of the *c* leaves as compared with that of the similar appearing but non-drought resisting *a* leaves might lead to a better understanding of the difference between these contrasting leaf types. Early development during a time of water shortage seems to engender drought enduring capacity, while attainment of maximum size and water content entails the loss of this capacity.

The degree of dormancy of the *c* leaves during the drought is of interest. With a water content about one-half their dry weight, metabolic activity must be definitely greater than that of air-dry leaves or seeds whose water content may be no more than one-tenth their dry weight. The appropriateness of the term "anabiosis" as applied to the condition of *Larrea* leaves (18) is thus questionable. The data on diurnal variation demonstrate that even in this state of minimum water content, *Larrea* leaves exhibit some diurnal varia-

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short periods of rain sufficient to result in water content increases, but not in growth. The time of occurrence and lengths of the rainy and dry seasons are different each summer. The general situation is as shown, however. June and July are usually the driest times in summer, and after early August rains another season of high temperatures and very low humidity is likely to occur. Even locally, however, there are apt to be conspicuous differences in the time and amount of precipitation.

[3] Water contents lower than half the dry weight are reported by THODAY (37) for some microphyllous South African ericads (*Passerina* spp) but these data are for whole shoots, leaves, and stems together. Twigs of *Larrea* always have a lower water content than the leaves (fig 4); and the older the stem, the lower the water content (unpublished data).

tion of the wc:dw ratio. No matter how much of this variation is due to dry weight change, fluctuation of the ratio implies activity. Experiments with moist chambers in the field (*q v.*) indicate that the transpiration rate must be low corresponding to a very slow water intake. The significant fact, however, is not that water content and the rate of transpiration are low, but that some activity, some transpiration does occur; the leaves do not become air-dry.

Nodal differences may be due to age, position, and history. In some cases, as when comparing *a1* and *a2* leaves, the difference in age from node to node is slight, a matter of a few days only. In other cases newly developed leaves (*a*) are contrasted with leaves of a former growth period (*c*) which may therefore be months older. In any case, the difference in leaf wc:dw ratio from node to node is of much smaller magnitude than in the same leaf from season to season. In other words, changes due to external conditions are greater than those due to internal development. That the water content increases with advancing age indicates that the amount of water held by a leaf increases faster than does the weight of dry substance. When the cells have attained maximum size, further increase in the ratio must be due principally to dry weight loss According to WALTER (40), the osmotic concentration also increases with age. This leads to the interesting deduction that the dry weight loss must be of the organic constituents, and more than enough to balance the increase in osmotic substances. A similar gradient in the percentage water content from younger to older leaves has been reported (41) for various plants, especially herbs. The second maximum (which according to YAPP and MASON occurs in buds, the minimum moisture content being in partially developed leaves) has been found in *Larrea* in only one test.

The reversed gradient of water content (decreasing with age) found during the four days of August 9–12, 1931, was exceedingly perplexing. The inconsistency with former results stimulated great care and effort to establish the true nature of the situation. A clue is afforded by some experiments, to be described later, with shoots in moist chambers. It was found that the youngest leaves have much the greatest water holding capacity, owing probably to their greater wall plasticity. During August 9–12 it may be supposed

that the plants had more than ample water to provide for transpiration. Their water content may thus have been conditioned principally by the extensibility of their cell walls. A descending curve of water content percentage from young to older leaves, even with constantly moist conditions, is reported to be the normal situation in many genera (27, 17, 22), but in *Larrea* is probably an unusual occurrence, being evidence of a superabundant water supply.

The characteristics of the diurnal changes are very evidently related to water conditions. The early day decrease and nocturnal increase are undoubtedly related to the changing rate of transpiration. On rare cloudy or rainy days the ratio does not sink so low. The diurnal minimum occurs late in the day more frequently in bushes of high water content, and earlier in the day in dry bushes. The interpretation of this situation may be that in drier bushes increasing water deficit *earlier in the day* reaches a point at which the colloids of the leaf tissues are in approximate equilibrium with their double environment, the taut tracheal sap and the desert atmosphere. Only a certain proportion of the total water content is seemingly subject to evaporation; this proportion is a larger quantity in bushes of higher water content; hence the turning point in the diurnal curve is reached later in the day in these bushes. Further, the magnitude of the diurnal change is related to the level of the water content. When the latter is only about 50 per cent of the dry weight and there has been no rain for months, the diurnal fluctuations are relatively slight, about 10 per cent of the early morning value; whereas when the water content is somewhat greater than the dry weight, the proportionate changes in the ratio may be more than 30 per cent, although more commonly about 20 per cent. Leaves of still higher water content show less proportionate diurnal change than leaves of medium water content; in the former, the water supply is evidently more nearly adequate to maintain an even water balance.

Probable changes of dry weight make the data relative to diurnal variations of the wc:dw ratio of only qualitative significance as regards water content. Diurnal changes of dry weight in semidormant drought-exposed leaves are probably less than for leaves of well watered plants in the relatively cool and humid environment of Cincinnati, where a small change in the dry weight of *Larrea* was demon-

strated. From three lines of evidence, (1) the Cincinnati experiments, (2) the correlation of water conditions with changes in the ratio, and (3) the lack of diurnal change of starch content in leaves of low ratio, the assumption seems justified that in the desert, especially in drier seasons, changes of the ratio are due much more to water content changes than to dry weight changes. During seasons of rain, however, when there is sufficient soil water to permit growth, dry weight as well as moisture undoubtedly undergoes diurnal fluctuations in direction opposite to that of moisture fluctuations, so that the ratio changes more than does the actual water content.

Diurnal variation of foliar moisture in *Larrea* is of about the same magnitude as for many other plants which come under WALTER's classification (40) of *euryhydric* species. These are unstable in percentage water content and sap concentration. Here belong such herbs as sunflower and potato (19), steppe plants such as *Plantago media* (11), and many desert plants (39, 25, 31, 19, 15). Contrasted with the euryhydric plants are those which are called by WALTER *stenohydric*, that is, having a very stable water balance. In the desert as in many other habitats euryhydric and stenohydric plants grow side by side.

A striking discrepancy appears (fig. 4) between the osmotic and the ratio values for the two experimental bushes Am and Bm. The level of the wc:dw ratio and the magnitude of its variation are greater for Am than for Bm; but the osmotic concentration of the sap is essentially the same in the two bushes, and follows conspicuously the same course. The difference between the two with respect to leaf size, vigor, and general appearance is paralleled by a difference in the wc:dw ratio, but not obviously in the osmotic concentration of the sap. These results emphasize the inadequacy of osmotic measurements alone in a study of water relations.[4]

It is impossible to assign definitely the reason for this discrepancy between osmotic and ratio values without supplementary data. The

[4] MALLERY (16) points out that the osmotic maximum occurs later in the day in bush Am (3 P.M.) than in Bm (1 P.M.), and that the variation in osmotic value is greater in Am. But these differences are certainly less striking than the close similarity of the osmotic curves for the two bushes, whereas the curves for the wc:dw ratio are not only of obviously different amplitude for the two bushes, but are on two distinct levels.

difference between the two bushes may be in water content, dry weight, or in both of these. Both chemical and anatomical investigations are required to give an adequate knowledge of water relations.

PISEK and CARTELLIERI (22) and OPPENHEIMER (21) have amplified their osmotic data with determinations of the percentage water content, and find in general a good correlation in the changes of the two quantities. In both these investigations, however, occasional discrepancies appear. For instance OPPENHEIMER reports that the stenohydric *Olea* undergoes more water loss than is indicated by the small rise in osmotic concentration; *Ceratonia* likewise may exhibit wilting phenomena but no concurrent increase in sap concentration. Chemical analyses of sap (30, 9) have shown that seasonal variations of osmotic value are due in some species (as *Ilex aquifolium, Hedera helix, Pinus sylvestris*) to sugar, in other species to both sugar and water (*Taxus baccata*), while in still others the variations may be due solely to water (*Buxus sempervirens*).

II. THE WC:DW RATIO UNDER EXPERIMENTAL CONDITIONS

THE WC:DW RATIO AT APPROXIMATE SATURATION.—To provide additional basis for comparison of naturally existing water content ratios, determinations were made of the values attained when shoots were inclosed in moist chambers. Some idea of the relation of the existing water content to that attained when the cells are distended to approximately their maximum size is afforded by use of the following method. Two branches are chosen, the moisture content of whose leaves by previous test has been found to be essentially the same. The leaves of one branch are picked for determination of the existing wc:dw ratio. The other branch is cut from the bush while the stem is held under water. The cut end of the severed shoot is kept under water while the whole is inclosed in a saturated atmosphere at a constant temperature for one or 24 hours, when the wc:dw ratio is determined. The data are not all strictly comparable because at times in the field it is necessary to deviate from the procedure just outlined. Two sources of error are thereby encountered:

1. The leaves picked for measurement of the original wc:dw ratio may not be strictly comparable with the leaves brought to saturation. A difference in the proportion of young to older leaves is espe-

cially to be avoided, because young leaves have a much higher water holding capacity.

2. The water content after 24 hours probably does not approach saturation to the same degree in all cases. The moist chambers used provide only an approximately saturated atmosphere. Some temperature changes are often unavoidable. Condensed moisture is sometimes visible as a film on glass or a mirror placed in the chamber. Moisture is never clearly discernible on the leaves, but for evaporation of any film that may have been present a period of exposure to dry air was often provided. An error in judgment as to the proper length of time for this would make some difference in the 24 hour ratio. The increases in the ratio are so great, however, that the errors involved are relatively insignificant.

Data relative to the saturation percentage water content, the amount of increase necessary to attain the 24 hour water content, and the rate of increase are presented in table IV and in figures 7 and 8.

Saturation water contents are found to be high above the naturally existing water contents, the latter being from less than one-third to only about three-fourths of the saturation values. The 24 hour ratio varies from bush to bush, with time of day, and with age of the leaf. Maximum values for the saturation ratio were obtained for bushes (Tb5 and Cinti, table IV) which had a predominance of young, actively enlarging leaves. While in general higher original ratios increase to higher saturation values, often it happens that of two bushes the one with the lower water content is closer to saturation. The existing ratio is no indicator of the saturation ratio.

The 24 hour *increase* is greater in leaves of low water content. Expressed in terms of the original ratio, the increase is very much greater in drier bushes (fig. 7). Thus, according to the data in table IV, wc:dw ratios 0.40–0.70 increase 2–300 per cent, while ratios originally over 1.27 increase less than 100 per cent in 24 hours. In all cases, however, a very considerable increase has been found. The increases represent from 23 to 77 per cent of the 24 hour ratio.

The *rate* with which water enters the stems of cut shoots is of special interest. Figure 8 shows that after the first few hours in the moist chamber the saturation value has already been quite closely

approached, and that thereafter the ratio increases very slowly to the 24 hour value. After 24 hours the ratio continues the very gradual increase, although at 48 hours in some cases a lower value than at 24 hours has been found. Derangement of the normal metabolism of

TABLE IV

CHANGE IN THE LEAF WC:DW RATIO OF CUT SHOOTS IN A MOIST CHAMBER

Bush	Date (1931)	WC:DW RATIO			24 HR. INCREASE IN % OF ORIGINAL W.C.	ORIGINAL W.C. IN % OF 24 HR. W.C.
		ORIGINAL	AFTER 1 HR.	AFTER 24 HR.		
Cj	July 23	0.47	....	1.76	274	26.7
Cj	July 15	0.58	....	1.73	196	33.3
Cj	July 18	0.59	1.08	1.72	193	34.1
mn	June 19	0.48	1.42	2.08	334	23.0
Bj	July 9	0.57	1.27	2.03	257	28.0
Bj	July 9	0.61	1.32	2.12	248	28.7
Bj	July 9	0.55	1.27	2.03	270	27.0
Bj	July 11	0.62	1.30	2 15	246	28.9
Ej	July 21	0.57	....	2.26	296	25.1
J	July 18	0.67	1.35	1.98	195	33.8
I	....	0.68	1.44	1.97	190	34.4
U₃₁	June 27	0.73	1.45	1.93	164	37.8
b	June 18	0.97	1.82	2.18	125	44.8
m₂	July 4	1.01	1.49	2.18	116	46.4
m₁	July 4	1.02	1.46	2.07	103	49.4
e	July 4	1.27	1.91	2.22	75	57.2
e	July 4	1.33	1.69	2.23	68	59.6
C	July 10	1.33	1.73	2.30	65	57.8
E	Sept. 4	1.57	....	2.76	76	56.9
E	Sept. 4	1.27	....	2.50	97	50.8
E	Sept. 4	1.17	....	2.26	93	51.7
E	Sept. 4	1.32	....	2.38	83	55.5
Am	Aug. 16	1.46	....	2 52	73	57.9
Am	Aug. 17	1.78	....	2.40	35	74.1
Am	Aug. 17	1.52	....	2.54	67	60.0
Am	Aug. 17	1.77	....	2.29	29	72.3
Am	Aug. 18	1.83	....	2.48	36	73.8
Am	Aug. 18	1.59	....	2.46	55	64.6
Tb₅	Aug. 12	1.78	....	3.02	70	59.0
Cinti	July, 1933	1.94	....	3.02	56	64.3

these cut shoots in a dark saturated atmosphere for periods longer than 24 hours makes doubtful the significance of 48 hour wc:dw values. The 1 hour percentage increase is roughly proportional to the 24 hour increase (fig. 7). Forty to 70 per cent of the total increase occurs during the first hour, and according to two tests (the

only ones made), 95 per cent of the 1 hour increase takes place within the first 15 minutes. Rapid increase of such magnitude in the wc:dw
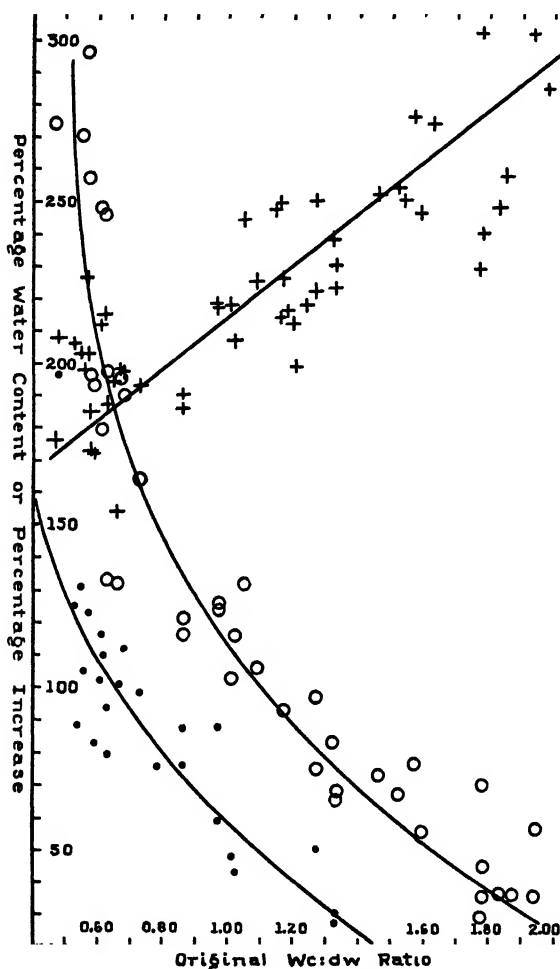
FIG. 7.—The 24 hour ("saturation") percentage water content (+) of leaves of bushes whose existing water contents ranged from 47 to 200%. For the same bushes the 24 hour *increase* (O) is shown as percentage of original value, and for some bushes the percentage increase after 1 hour (●). Curves drawn merely to indicate general regions in which the points fall.

ratio must be due principally to increase in water content; dry weight could not diminish so greatly in 15 minutes.

Further evidence in regard to how much dry weight may occur in leaves of severed shoots while in the moist chambers was obtained by the half-leaf method already described. The results of five experiments indicate that the change in water content is about eight times that of the dry weight.
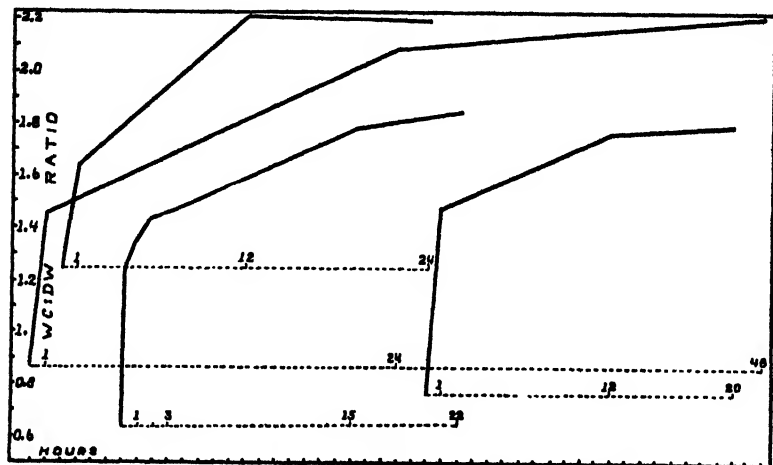
FIG. 8.—The wc:dw ratio of leaves of four shoots in a moist chamber at various time intervals after cutting from the plant and putting the stems in water.

Changes in leaves of the cut shoots during 24 hours in a moist chamber averaged   5.05% loss in dry weight

41.2 % increase in water (mg.)
46.1 % increase in wc:dw
14.5 % increase in water as per-
centage of fresh weight.

These experiments were conducted at the University of Cincinnati. In Arizona, higher temperatures may have led to greater change in dry weight, but probably not more than as much as to account for 15 per cent of the 24 hour increase in the ratio.[5]

EFFECT OF ELIMINATING TRANSPIRATION.—In another series of experiments with shoots inclosed by moist chambers, the stems were not cut and put in water but were left intact, the moist chambers

[5] A single test of the temperature effect in Tucson indicated no appreciable difference between 28° and 40° C. in the rate of increase of the wc:dw ratio.

being adjusted to the shoots in the field. Any increase in water content under these circumstances must be from the existing supply in the stems, roots, and soil. This type of experiment, carried out according to the procedure outlined below, proved to be a very simple and effective way of determining roughly the relative influences of transpiration and of insufficient water supply on the water balance.

A bell jar furnished with one or more wicks of paper or cloth toweling extending from the top down into a shallow pan of water is adjusted over the selected shoot. The pan of water rests on a support covered with one or more layers of waterproofed fabric which is fitted snugly around the stem of the shoot and up around the base of the bell jar, and made tight with elastic tubing. Two coats are fitted over the bell jar: one of thick paper felting for heat insulation, and an outside coating of glistening white paper to minimize sunlight absorption. The temperature within the chambers does not differ significantly from the external temperature. Dew formed in the chambers as a result of temperature fluctuation has always to be evaporated before leaves are picked for determination of wc:dw ratio. To outward appearance, shoots of *Larrea* which have been in a moist chamber even as long as 2 or 3 days appear normal in every respect. The leaves, however, doubtless suffer some loss of their substance owing to continued respiration in the absence of photosynthesis, so that the wc:dw ratio may be expected to increase even independently of any increase in water content.

In contrast to the experiments with cut shoots, no sudden large increase in water content takes place. Indeed, except in bushes k31, x, y, and z (table V), the increase in the wc:dw ratio as a result of the use of moist chambers in the field was small, and in many cases may have been due to dry weight decreases.

When only one shoot of bushes t31, h31, and bl was inclosed by a moist chamber, similar diurnal fluctuations were shown by both exposed and experimental leaves, although the latter were protected from transpiration. This implies that protected leaves lost water during the day to nearby exposed transpiring leaves. Transpiration increases the osmotic concentration of the sap of exposed leaves over that of protected leaves so that the former can draw on the protected leaves for part of their water supply. This translocation of water

from one shoot to another indicates a deficient soil water supply, and did not occur when there was ample, as for bushes g, bs, and k31 (table V). Bushes x, y, and z (fig. 9) show both situations. By com-

TABLE V

CHANGE IN LEAF WC:DW RATIO OF INTACT SHOOTS IN A MOIST CHAMBER

BUSH	DATE	NUMBER OF WC:DW DETERMINATIONS	VARIATION LIMITS OF WC:DW RATIO		24 HR. "SAT." WC:DW	CHARACTERISTICS OF THE WC:DW RATIO OF THE PROTECTED LEAVES, ETC.
			EXPOSED	PROTECTED		
Ej	July 15–28 1931	32	0.57–0.61	0.58–0.66	1 70	Whole bush covered July 21; increase over control, and variations slight
Aj	July 9–13 1931	10	0.60–0.62	0.65–0.66	1.87	Readings at 3 P.M.
t31	June 26–28 1931	20	0.74–0.83	0.75–0.86	....	Follows control
g	June 14–15 1930	10	0.74–0.83	0.90–0.93	1.93	Does not follow control; readings at 5:30 A.M. and 2:30 P.M.
bs	July 27–30 & Aug. 3–4 1930	18	0.67–0.90	0.80–1.02	....	Does not follow control; no midday drop
h31	July 2–11 1931	18	0.83–1.02	0.94–1.07	1.77	Follows control, when not entirely covered
k31	July 1–5 1931	15	0.92–1.01	0.98–1.37	....	Does not follow control; marked increase when entirely covered
bl	June 17–18 1931	14	0.90–1.09	0.88–1.09	2.18	Follows control
x, y, & z	Aug. 26–Sept. 5 1931	66	0.99–1.50	1.01–2.16	2.45	Follows or not, depending on conditions (see fig. 9)

parison of the curves for x and y for August 26–27 with those of all three bushes for September 1–2, the effect of rains which fell prior to September 1 is indicated. It is interesting to compare also bushes g and t31 (table V), exposed shoots of which had the same leaf water content and diurnal fluctuation, 0.74–0.83. The water content of the protected leaves of bush h31 decreased at midday just as did the exposed control, while the wc:dw ratio of protected leaves of bush g
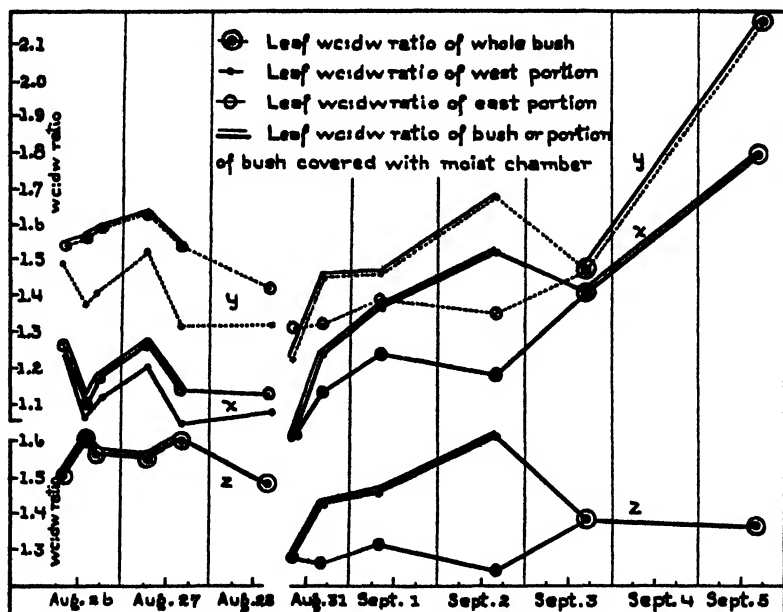
FIG. 9.—Variations in wc:dw ratio of leaves of bushes x, y, and z when partly and when completely protected from transpiration by the use of moist chambers in the field.

remained about constant or increased slightly. The wc:dw ratio of the exposed shoots alone fails to indicate the better water supply of bush g.

The effects of water translocation can be avoided by covering entire bushes. This was done for the small bushes Ej, h31, k31, x, y, and z. Even when completely covered, the resulting increase in the wc:dw ratio for bushes Ej, h31 (table V) and for bush z, Aug. 26–27 (fig. 9), was surprisingly small. The very slow reduction of the water tension, when transpiration is held at minimum by means of a moist

chamber, is convincing evidence of a deficient water supply. Transpiration, having once reduced the water content to a low level, is relatively much less important than the diminishing water supply in maintaining a low wc:dw ratio in these bushes. It is to be emphasized that this is the usual situation; not transpiration, but insufficient soil water is what maintains the low moisture content in *Larrea* leaves.

On the other hand, when there is enough water for growth, transpiration is very active in restricting the wc:dw ratio. Thus Sept. 3-5, when all of the shoots of bushes x and y (fig. 9) were covered, a comparatively rapid increase took place while bush z, entirely exposed, suffered a slight decrease. Here again the inadequacy of the wc:dw ratio of exposed leaves alone to indicate the situation is seen by comparing the data for h31 and k31 (table V). The wc:dw ratio of the leaves of both bushes is about the same. The moist chamber experiments, however, show that water is relatively more available to the leaves of bush k31 which when covered exhibited a markedly increased ratio.

General discussion.—The data presented in this section demonstrate that of two bushes having the same water content, one may be much further from its saturation water content or may have a significantly smaller water reserve than the other. The use of moist chambers in the field on intact plants may prove to be a valuable method of investigation of the water balance of other species.

The amount of deviation of the natural wc:dw ratio from the saturation value is related somewhat to the capacity of a plant to endure drying, but is clearly not to be taken as an indicator of this capacity. The saturation wc:dw ratio is never attained in nature; it is not a constant; and it is higher than the existing ratio partly because of dry weight changes (39). The inconstancy of the saturation water content is easily understood, for the total water capacity of a cell depends first upon the varying difference between its vapor tension and that of its environment, and second, upon the extensibility of its walls. The relatively high capacity shown by young leaves is no doubt due to the greater plasticity of their walls.

The great rapidity of water intake when shoots are cut under water (fig. 8) evidently indicates that a high tension normally exists

in the hydrostatic system of the shoots. The elimination of transpiration from intact shoots does not immediately relieve this high tension. The highest ratios attained by leaves on intact shoots, even though these are surrounded by a nearly saturated atmosphere, are still well below the saturation ratios (table V).

TABLE VI

DIFFERENCE BETWEEN EXISTING AND SATURATION WC:DW RATIO
IN PERCENTAGE OF SATURATION VALUE

REFERENCE	REGION	HABITAT	PLANT	PERCENTAGE DIFFERENCE ("DEFICIT")
(11)	South Russia	Swamp Meadow Steppe	Alisma plantago Rumex confertus Plantago major	0 5-7 7 4-9 19-40
(26)	Arizona	Desert	Encelia farinosa	-50
(25)	Arizona	Encinal	Quercus emoryi Arctostaphyllos pungens	28-29 17-27
(37)	South Africa	Desert	Passerina spp.	-55
(31)	Egypt	Desert	Non-succulent plants Mesembryanthemum	35-56 19
(33)	Hungary	Alkali steppe Baltic sea-coast	Achillea millefolium Epilobium montanum Calluna vulgaris	4-19 11-16 -23
(34)	Swedish Lapland	Arctic tree limit	Shrubs and herbs	4-10
(22)	Mid-Europe	Alpine tree limit Shade Sun	Ericaceae spp. Mercurialis perennis Coronilla varia	"0"-30 4-12 1-33
(21)	Jerusalem	University campus	Ficus elastica Rosmarinus officinalis	-16 30-65
RUNYON	Arizona	Desert	Larrea tridentata	23-77

In table VI are given approximate values for the percentage difference between the existing and saturation wc:dw ratios for various plants. The methods used by the investigators have differed, so that the data are only roughly comparable. The terms water deficit and saturation deficit have often been used to designate this difference.

It is seen that *Larrea* may endure a water content further from its maximum water holding capacity than any other plant reported.

Summary

1. From various lines of evidence it is shown that the dry weight of *Larrea* leaves undergoes diurnal fluctuations when there is enough water for growth; but under the usual desert conditions the dry weight probably changes very slowly, water conditions being chiefly responsible for the fluctuations of the wc:dw ratio. However, the usual disregard of dry weight changes in literature pertaining to percentage water content, and the use of the fresh weight basis for expressing the data, have often led to erroneous interpretations.

2. The amount of moisture relative to dry weight in leaves of *Larrea* differs with age, position on the stem, season, habitat, and time of day.

3. Apart from seasonal influences, water content increases with the age of the leaves, except during rare periods of ample soil water, when the younger more plastic leaves have the higher water content.

4. During periods of growth the water content of the foliage is not lower than that of many mesophytic tree leaves: between one and two times the dry weight. During severe drought the leaves may endure a water content less than half the dry weight, a value lower than that reported for any other seed plant (except air-dry plants or plant parts).

5. Leaves at adjacent nodes, although practically indistinguishable in appearance and structure, may differ greatly in physiological capacity, depending upon the water conditions existing during their development. Three leaf types, *a*, *b*, and *c*, may be recognized. The *a* type, most abundant, has a comparatively high water content, and very little or no drought resistance. The *b* type is intermediate in all respects to *a* and *c*. The *c* leaves are resistant to drought, have a lower water content during their expansion from the bud, and a longer life span than the *a* leaves.

6. Development during a time of water shortage seems to engender drought enduring capacity, while attainment of maximum size and water content entails loss of this capacity.

7. The drought enduring *c* leaves have two periods of enlargement,

separated by a season of drought-induced dormancy. This dormancy is not complete, as is shown by the facts that the leaves are not air-dry and that a slight diurnal variation of the wc:dw ratio occurs.

8. In general, osmotic changes follow in a reciprocal manner the diurnal water content changes, but the latter may disclose differences not evident in the osmotic measurements alone.

9. Large and very rapid increases in the water content of the leaf occur when the stems are cut and allowed to remain under water, the leaves being inclosed by a moist chamber. This implies a high tension in the tracheal sap.

10. The saturation water content is higher above the naturally existing water content for *Larrea* leaves (*b* and *c* types) than for any other species for which data have been found in the literature.

11. By following the changes of the wc:dw ratio of leaves of bushes partly or completely covered with moist chambers, the adequacy of the water supply to the leaves may be judged.

12. Under usual conditions a deficient water supply rather than transpiration is the crucial factor limiting the moisture content; while after rains the rate of transpiration may be very high and the chief cause of the considerable difference between the saturation and existing water contents.

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LITERATURE CITED

1. ASHBY, E., Transpiratory organs of *Larrea tridentata* and their ecological significance. Ecology 13:182–188. 1932.
2. BACHMANN, F., Studien über Dickenänderungen von Laubblättern. Jahrb. Wiss. Bot. 61:371–429. 1922.
3. BAKHUYZEN, H. L. VAN DE S., Studies upon wheat grown under constant conditions. Plant Physiol. 3:1–30. 1928.
4. BEWS, J. W., and AITKEN, R. D., Researches on the vegetation of Natal. Ser. II Bot. Survey So. Africa Memoir 8:5–34; 41–65. Pretoria. 1925.

5. CALDWELL, J. S., The relation of environmental conditions to the phenomenon of permanent wilting in plants. Physiol. Researches 1:1–56. 1913.

6. CLARK, A. W., Seasonal variation in water content and in transpiration of leaves of *Fagus americana, Hamamelis virginiana,* and *Quercus alba.* Contrib. Bot. Lab. Univ. Pennsylvania 4:105–143. 1919.

7. DENNY, F. E., Bases for calculations in measuring changes in leaves during the night. Contrib. Boyce Thompson Inst. Plant Res. 5:181–194. 1933.

8. GILBERT, B. E., and ADAMS, W. L., Moisture fluctuations in extracted plant solutions and in leaf tissue. Plant Physiol. 4:529–536. 1929.

9. HUBER, B., Die Wasserumsatz in der Pflanze. Fortschr. Bot. 2:145–158. 1933.

10. HURD-KARRER, ANNIE M., and TAYLOR, J. W., The water content of wheat leaves at flowering time. Plant Physiol. 4:393–397. 1929.

11. ILJIN, W. S., Der Einfluss des Wassermangels auf die Kohlenstoffassimilation durch die Pflanzen. Flora 116:360–378. 1923.

12. KNIGHT, R. C., Further observation on the transpiration, leaf water content, and wilting of plants. Ann. Bot. 36:361–383. 1922.

13. KÔKETSU, R., Variations in the water content of leaves as related to the wilting of plants. Jour. Dept. Agr. Kyushu. Imp. Univ. 2:93–116. 1928.

14. KRAUS, E. J., and KRAYBILL, H. R., Vegetation and reproduction with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149. 1918.

15. LIVINGSTON, B. E., and BROWN, W. H., The relation of the daily march of transpiration to variations in the water content of foliage leaves. BOT. GAZ. 53:309–330. 1912.

16. MALLERY, T. D., Changes in the osmotic value of the expressed sap of leaves and small twigs of *Larrea tridentata* as influenced by environmental conditions. Ecological Monographs 5:1–35. 1935.

17. MARSHALL, R., Water relations of seedling conifers with special reference to wilting. Ecological Monographs 1:37–97. 1931.

18. MAXIMOV, N. A., The plant in relation to water. A study of the physiological basis of drought resistance. English transl. by R. H. YAPP. Allen & Unwin, London. 1929.

19. ———, and KRASNOSSELSKY-MAXIMOV, J. A., Wilting of plants in its connexion with drought resistance. Jour. Ecol. 12:95–109. 1924.

20. MOINAT, A. D., Available water and the wilting of plants. Plant Physiol. 7:35–46. 1932.   •

21. OPPENHEIMER, H. R., Zur Kenntnis der hochsommerlichen Wasserbilanz mediterraner Gehölze. Ber. Deutsch. Bot. Ges. 50a:185–245. 1932.

22. PISEK, A., and CARTELLIERI, E., Zur Kenntnis des Wasserhaushaltes der Pflanzen. I. Sonnenpflanzen. Jahrb. Wiss. Bot. 75:195–251. 1931. II. Schattenpflanzen. *ibid.* 643–678. 1932. III. Alpine Zwergsträucher. *ibid.* 79:131–190. 1933.

23. RUNYON, E. H., The organization of the creosote bush with respect to drought. Ecology 15:128–138. 1934.

25. SCHRATZ, E., Vergleichende Untersuchungen über den Wasserhaushalt von Pflanzen im Trockengebeit des südlichen Arizona. Jahrb. Wiss. Bot. 74: 153–290. 1931.

26. SHREVE, E. B., Factors governing the seasonal changes in transpiration of *Encelia farinosa.* BOT. GAZ. 77:432–439. 1924.

27. SLUYTER, F., Untersuchungen über den Wassergehalt in den Laubblättern. Inaug. Diss. Kiel. 1900.

28. STEFANOFF, B., Studien über den Zustand und die Schwankungen des Wassergehaltes in den Blättern und Zweigen einiger Holzpflanzen. Forstw. Centralbl. 53:784–799. 1931. (Abstract in Biol. Abst. 7:no. 7892.)

29. ———, and STOICKOFF, J., Ueber den Wasserhaushalt der Holzpflanzen. Jour. Ecol. 20:89–104. 1932.

30. STEINER, M., Zur Chemismus der osmotischen Jahresschwankungen einiger immergrüner Holzgewächse. Jahrb. Wiss. Bot. 78:564–622. 1933.

31. STOCKER, O., Der Wasserhaushalt ägyptischer Wüsten- und Salzpflanzen. Bot. Abh. 13. G. Fischer, Jena. 1928.

32. ———, *in* Tabulae Biologicae 5 Botanik. W. Junk, Berlin. 1929.

33. ———, Das Wasserdefizit von Gefässpflanzen in verschiedenen Klimazonen. Planta 7:382–387. 1929.

34. ———, Transpiration und Wasserhaushalt in verschiedenen Klimazonen. I. Untersuchungen an der arktischen Baumgrenze in Schwedisch-Lappland. Jahrb. Wiss. Bot. 75:494–549. 1931.

35. ———, II. Untersuchungen in der ungarischen Alkalisteppe. *ibid.* 78:751–856. 1933.

36. THODAY, D., A critical examination of Sachs' method for using increase of dry weight as a measure of carbon dioxide assimilation in leaves. Proc. Roy. Soc. London B. 82:1–55. 1909.

37. ———, Behavior during drought of leaves of two Cape species of *Passerina* with some notes on their anatomy. Ann. Bot. 35:585–601. 1921.

38. TRELEASE, S. F., Incipient drying and wilting as indicated by movements of coconut pinnae. Amer. Jour. Bot. 9:253–265. 1922.

39. VASSILJEV, I. M., Über den Wasserhaushalt von Pflanzen der Sandwüste in südöstlichen Kara Kum. Planta 14:225–309. 1931.

40. WALTER, H., Die Hydratur der Pflanze und ihre physiologischokologische Bedeutung (Untersuchung über den osmotischen Wert). G. Fischer, Jena. 1931.

41. YAPP, R. H., and MASON, U. C., The distribution of water in the shoots of certain herbaceous plants. Ann. Bot. 46:159–181. 1932.

GROWTH OF FRAGMENTS OF EXCISED
ROOT TIPS[1]

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(WITH SIXTEEN FIGURES)

The experiments on the cultivation of fragments of root tips de-
scribed in this paper were undertaken with two particular objectives
in mind: first, to determine what types of fragments of root tips
grow in pure culture; second, to observe the development of these
fragments in the hope of learning by experiment something of the
controlling factors.

Materials and methods

In the major portion of the experiments, root tips of corn (*Zea
mays*) were used. Some experiments were performed also with root
tips of wheat (*Triticum vulgare*) and of peas (*Pisum sativum*). The
methods used in cultivating the root tips under sterile conditions
were similar to those employed by ROBBINS (7, 8) and by ROBBINS
and MANEVAL (9, 10). In securing the root fragments, the grains or
seeds were surface sterilized with calcium hypochlorite or with mer-
curic chloride in 50 per cent alcohol, washed with sterile water, and
germinated on sterile 1 per cent water agar in petri dishes. When the
roots had reached a length of 1–2 cm., pieces were cut by a sterile
knife and transferred to a sterile medium containing mineral salts,
2 per cent glucose, and 0.75 per cent agar in 150 cc. Erlenmeyer
flasks or in petri dishes. The knives used were made by fixing frag-
ments of safety razor blades or suitably ground sewing needles in an
aluminum handle. The roots were fragmented for the most part
without visual aids. In some cases a binocular microscope was used.
Contaminations were few.

Three agar media were used. Medium I contained calcium ni-
trate, 50 p.p.m.; magnesium sulphate, 10 p.p.m.; potassium dihy-

drogen phosphate, 10 p.p.m.; ferric chloride, 1 p.p.m.; glucose, 2 per cent; agar, 0.75 per cent. Medium II was medium I containing, in addition to the constituents given, sodium borate, 0.1 p.p.m.; manganese chloride, 0.1 p.p.m.; zinc chloride, 0.1 p.p.m. Medium III was prepared by placing half a sterile corn grain which had germinated three days at 28° C. in the center of a plate containing agar medium II. The freshly cut surface of the grain was pressed into the agar. The plates were then placed in the dark for five days to allow diffusion from the germinated grain to occur. At the end of five days the half grain was removed and the fragment of the root tip was placed on the agar plate.

Preliminary experiments

Grains of Longfellow flint corn were germinated under sterile conditions. A terminal piece of the root tip less than 3 mm. in length was removed by a cut across the root. Although the cuts were intended to be perpendicular to the long axis of the root they were in some cases more or less oblique. These short tips were transferred to a sterile agar medium in petri dishes and incubated in the dark at room temperature. Using a compound microscope and ocular micrometer, the original length of the fragment was measured. Daily measurements of growth were made and final lengths and observations were taken after 7 to 18 days.

The original length of the tips ranged from 0.27 to 2.62 mm. The shortest root tip which grew was originally 0.32 mm. long and reached on medium II a length of 3.8 mm. in 7 days. Most of the tips less than 0.4 mm. in length did not grow, however, only two out of eleven less than 0.4 mm. increasing in length. The greater proportion of those tips from 0.4 to 0.49 mm. long also failed to grow; 19 out of 50 in this group growing. The best growth on medium II in this group was a root tip originally 0.45 mm. long which grew to 15 mm. in 10 days. When the original length of the root tip was 0.5 mm. or more, the majority of the tips grew. From table I it is obvious that excised corn root tips under the conditions of these experiments must be more than 0.5 mm. in length for growth to occur in the majority of cases; but to secure growth in all cases the tips should be 0.9 mm. or more in length.

No difference in the effects of the three media on the percentage of root tips which grew in the different groups was noted. For final lengths medium III was superior to medium II and medium II was superior to medium I. A discussion of the effect of these media on the amount of growth will be given in a separate publication.

Corn root tips such as were used in these experiments consist of two main parts, the root cap and the meristem. How much of a particular excised root tip is composed of root cap cannot be stated ex-

TABLE I

PERCENTAGE OF ROOT TIPS OF LONGFELLOW FLINT CORN WHICH GREW ON AGAR MEDIUM; ROOT TIPS GROUPED ACCORDING TO ORIGINAL LENGTHS

ORIGINAL LENGTH (MM.)	NUMBER OF ROOT TIPS	PERCENTAGE WHICH GREW
0.20–0.29	3	0
0.30–0.39	8	25
0.40–0.49	50	38
0.50–0.59	31	86
0.60–0.69	44	93
0.70–0.79	25	84
0.80–0.89	30	96
0.90–0.99	3	100
1.00–1.99	8	100
2.00–2.99	84	100

actly. Microscopic examination of the excised tips on the agar medium in the culture dishes does not permit accurate measurement of the root cap as distinct from the meristem, and all measurements given are "over all" lengths. The length of the root cap varies with the individual root and is affected by the root cap cells pushed away in manipulation. Measurements of prepared sections of 15 corn root tips similar to those used in these experiments showed the root cap to vary in length from 0.26 to 0.38 mm., averaging 0.33 mm. The failure of a considerable proportion of those root tips less than 0.5 mm. in length to grow does not mean, therefore, that a piece of the root meristem 0.5 mm. or more in length is necessary to secure growth. If the shortest fragment which grew (0.32 mm.) had a root cap equal in length to the shortest root cap measured, it would contain a meristem 0.06 mm. long.

In figure 1 the percentage of root tips which grew is plotted against the original length of the tips. On the same figure a root tip is drawn showing the average length of the root cap. If averages only are considered, the average length of the meristem when 50 per cent of the root tip fragments grew was 0.15 mm., and when 75 per cent of them grew it was 0.19 mm.
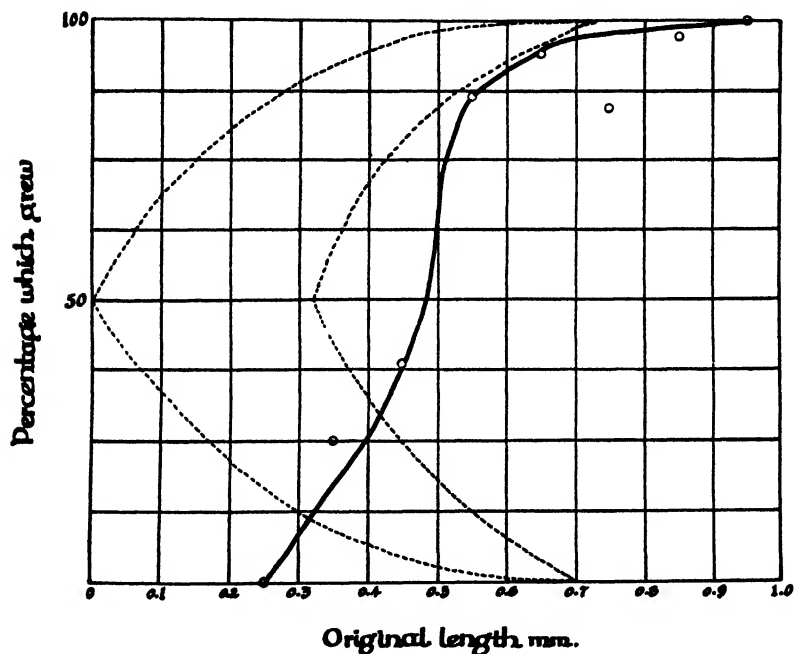
FIG. 1.—Relation between original lengths of excised root tips of corn and percentage which grew under sterile conditions on nutrient medium. Dotted lines show average length of root cap.

As growth occurs in a root when attached to the plant, the cells at the apex of the meristem remain embryonic while the older parts of the meristem differentiate in an orderly manner into the epidermis, cortex, and stele. In these experiments, in which isolated pieces of root meristem were grown, tips originally 1 mm. or more in length developed into normal roots; and most of those less than 1 mm. long, which increased in length, also formed normal roots. At the end of the experiment, the isolated piece of root meristem had formed a

root consisting of a root cap, apical meristem, epidermis, cortex, and stele. Root hairs and branch roots had been produced.

While it is not possible from these experiments to state categorically the minimum length of meristem in an excised corn root tip necessary for normal development, the following tentative conclusions may be offered. It appears that the excised root cap including the calyptrogen does not under the conditions of these experiments form a normal root. It is probable that a terminal piece of the meristem 0.1 mm. in length will form a normal root.

Normal polarity was evidenced by excised corn root tips 0.5 mm. or less in length. These tips measured less in the direction of the long axis of the root than they did in a direction across the root, and were composed of the root cap and a bit of the apical meristem. In such pieces growth occurred in the direction of the long axis of the original root and the cells elongated in the same direction as they do in the normal root.

An occasional root tip less than 1 mm. long developed into a root, normal in all respects except that the apical meristem did not remain embryonic but differentiated into elongated parenchyma cells. In such cases the tip of the root contained no embryonic cells at the termination of the experiment (fig. 2).

Furthermore, occasionally the shorter pieces of meristem did not differentiate epidermis, cortex, and stele, but developed into a flat filament (sometimes two) the cells of which resembled cortical and epidermal cells. Such filaments lacked a stele and embryonic region and were usually tightly coiled.

Many of the shorter root tips, especially those less than 0.5 mm. in length, did not elongate but formed a mass of cells, circular in outline, on the surface of the agar. The diameter of this mass was rarely more than two or three times the original length of the tip. The mass consisted of a central core of cells surrounded by a jelly-like matrix in which root cap cells were imbedded. In some cases the original tip consisted of root cap only, in others a bit of the meristem also was included. A typical development of this type is shown in figure 3. No information as to whether cell division occurred in the formation of these masses is at hand. It is probable that they resulted from the enlargement of individual cells and the gelatinization of cell walls.
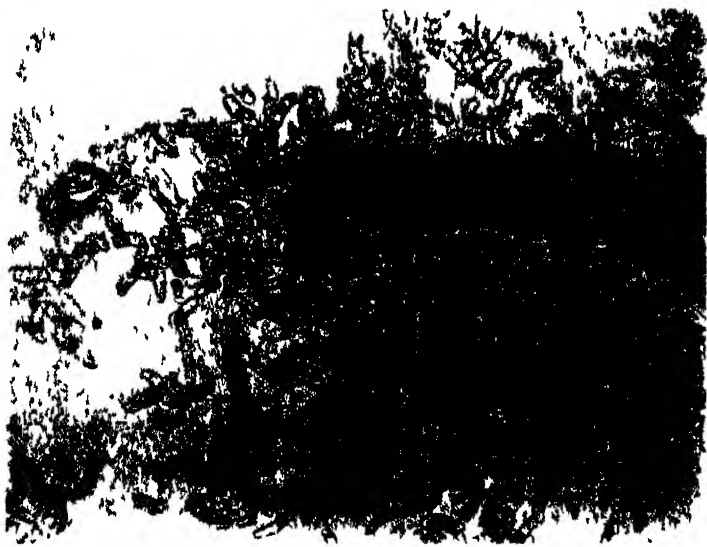
FIG 2

FIG 3

FIGS 2, 3 —Fig 2, tip of developing excised corn root tip originally 0 48 mm in length Some root cap cells show an extrusion of protoplasm Note that tip is not meristematic but is composed of elongated parenchyma cells Fig 3, development of excised corn root tip originally 0 48 mm long Note central core composed largely of root cap cells and scattered root cap cells imbedded in jelly-like matrix

Microscopic examination showed that the outer layers of the cell walls of the root cap cells had gelatinized. This gelatinization probably does not include the middle lamella, as bits of a delicate network were visible here and there in the gelatinous mass. These were interpreted as middle lamellae left when adjacent parts of the cell wall gelatinized. The root cap cells do not migrate but are entirely passive in their separation one from another, which is due to the swelling of the gelatinized cell wall layers between them. This is probably the explanation for the "migration" of root cells reported by CHAMBERS (1). The separated cells frequently became half-moon shaped and an extrusion of protoplasm was often observed on the convex side of the cell, presumably through a pore formed in the wall (fig. 2).

Why cells at the apex of the root meristem should retain their embryonic character, while a short distance from the apex they lose it and form the various permanent tissues of the root, is a question for which as yet we have no adequate answer. What are the conditions which determine the existence of a meristem and conversely what determines the differentiation of cells? In the preliminary experiments just described, some of the short pieces of the meristem did not retain their meristematic character, although all the tissues normally formed in a root were differentiated; and other pieces did not form all the permanent tissues normally found in the root. Why should pieces of the apical meristem of a corn root tip respond in these fundamentally different ways? Are the differences in their responses determined by the treatment of the fragment (injury from cutting, culture medium), or by the amount or kind of tissue included?

The preceding experiments were concerned with short root tips which had been removed by a cut across the root, perpendicular to the long axis of the root in most cases but oblique in some. When a tip 0.5–1 mm. in length is removed from a corn root by a transverse cut, the excised piece includes the initial cells at the tip of the embryonic region and a portion of the three histogens present in the meristem (plerome, periblem, and dermatogen). If growth took place it might be anticipated, since the histogens from which the various permanent tissues develop are all present, that a complete

root with a differentiated stele, cortex, and epidermis would be formed. Even in such a case, however, it is possible that too small a piece of the apical meristem would not grow under the conditions of the experiment, or, if it did, all of it would differentiate and no embryonic region would remain at the end of the experiment.

When a root tip is cut obliquely, the fragment removed may or may not include the initial cells and may lack one or more of the histogens. The development of such fragments might conceivably be very different from that of a bit of the meristem which included the initial cells and some of each of the three histogens.

To explore the relation which might exist between the character of the tissues included in a root tip fragment and its development, root tips 1–2 mm. in length were fragmented by longitudinal, oblique, or transverse cuts and the fragments cultivated on a nutrient medium. To cut fragments from a seedling root tip 1–2 mm. in length which had previously been removed from the root was found to be extremely difficult. Fragmentation was simplified by making the proper cuts before the tip was removed from the root. After fragmentation, the pieces were placed on medium I in petri dishes or in 150 cc. Erlenmeyer flasks and grown in the dark. After one to three weeks, the resulting structure was transferred to a drop of lactophenol containing cotton blue and acid fuchsin (4). The cleared root fragments, in which differentiated cells stained red and embryonic cells a deep blue, were then examined microscopically. The results of the experiments to be described show that a fragment of the apical meristem developed normally if it included a portion of the plerome not less than 0.1–0.2 mm. in length and equivalent to between one-quarter and one-half of that found at the root apex. If less than this amount of the plerome was included or if periblem and dermatogen alone were used, a complete root was not formed.

Further experiments

ROOT TIPS FRAGMENTED BY MEDIAN LONGITUDINAL CUTS

Nine root tips of corn, varying from 1 to 2 mm. in length, were cut longitudinally into two parts, nearly equal in size. Both pieces of six of these sets of fragments developed into complete normal roots. In one week the pieces originally 1–2 mm. long grew to lengths of 16–18

mm. and each produced several secondary roots. Such a set of fragments is illustrated in figure 4. In the upper part of the figure is a diagram showing how the tip, measuring 1.2 mm., was cut into two equal pieces, *A* and *B*. The development of these two pieces is shown diagrammatically in the lower part of the figure at *A* and *B*. The base of each of the developed fragments contains stele (shaded) on one side but no cortex; toward the tip the stele is more nearly central, until eventually the root is entirely normal.

In the original half root tip the plerome is exposed on one side. This exposure in this way amounts to changing its relative position in the root, since in the half root it no longer occupies a central position. If the relative position of the plerome in the original root is a factor of importance in determining its differentiation into the elements of the stele, we might expect that the exposed plerome in a half root tip, formed by a medium longitudinal cut, would differentiate into epidermis and cortex instead of into stele. However, in the basal portions of the roots which developed from these half root tips the stelar tissue was located on one side in the position occupied by the plerome in the original half root (fig. 4). The change in relative position had no noticeable effect upon the differentiation of the basal and older portion of the plerome.

On the other hand, continued growth resulted in the formation of a complete root, not a half root. This indicates that cells at the tip of the embryonic region are not fixed in the potentialities of their development, as are the older cells of the plerome, but are capable of development into any of the various elements of the root. It is probable that this group of cells is not limited to the initial cells as it does not seem possible that a razor, in dividing a root tip into halves, would separate the group of initial cells into two groups so that each half of a root tip would possess some of them. In any event, it is clear that the fate of the plerome cells is fixed early in their development in such fashion that change in relative position does not materially modify their development. Just how early determination occurs cannot be stated, but judging from the length of the basal portion of the root which contains stele on the outside, it would appear to occur within 0.2 or 0.3 mm. of the apex of the root meristem.

Of the pairs of fragments which did not develop into normal

roots, the members of one pair had increased in length from 1 to 8 mm. in five days. Some stele was formed, but at the end of the experiment no apical meristem was present in either piece; all of the
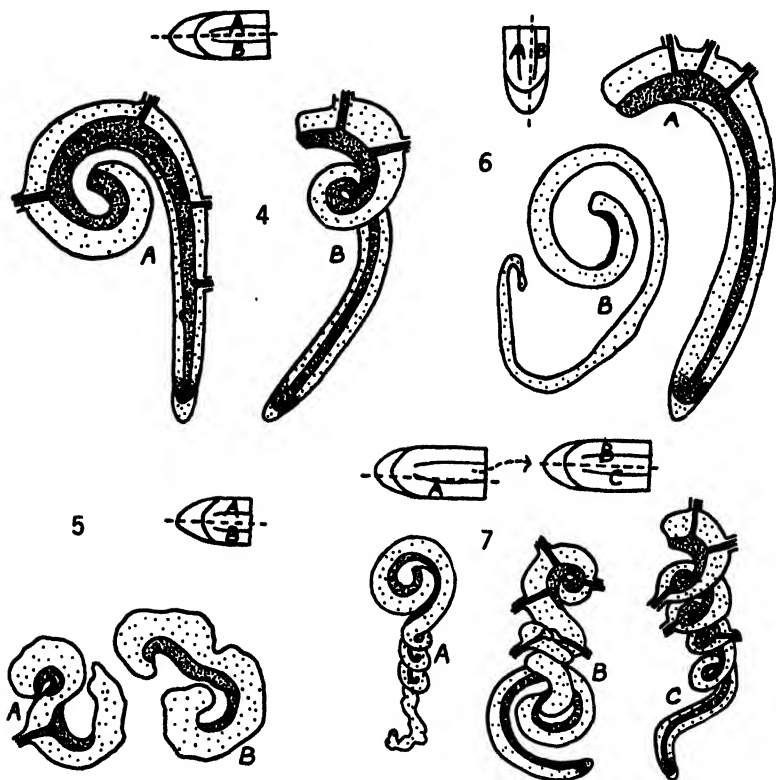
Figs. 4–7.—Fig. 4, development of fragments of corn root tip originally 1.2 mm. long and cut longitudinally into equal parts; each piece developed into a normal root but basal portion has stele on one side. Fig. 5, development of fragments of corn root tip originally 1.0 mm. long and cut longitudinally into equal parts by a coarse razor (Gitsnife). Neither piece developed into a normal root although stelar tissue was differentiated and branch roots formed. Fig. 6, development of fragments of corn root tip originally 1.0 mm. long and cut longitudinally into unequal parts. Larger piece developed into a normal root although with stele on one side at the base; smaller piece formed some stele at base but lacked root cap and apical meristem. Fig. 7, development of fragments of corn root tip originally 2.0 mm. long and cut longitudinally into unequal parts, the larger piece then cut longitudinally into equal parts as shown in diagram. A formed some stele but lacked root cap and apical meristem; B and C developed into normal roots although with stele on one side at base.

meristem had differentiated into elongated parenchyma cells. This set of fragments is shown diagrammatically in figure 5.

In another pair, the root fragments were grown for two weeks and the lengths of the pieces increased from 1 mm. each to 8 and 16 mm. respectively. Each piece contained some stele. Neither piece possessed an apical meristem.

Another set of fragments grew from 1.5 to approximately 15 mm. Both were tightly coiled, the cut surface forming the inside of the coil and no apical meristem was present.

These three sets of fragments in which all of the meristem differentiated were prepared by using a "Gitsnife" instead of the thinner blade of the Gillette type which was used in the other experiments. It is probable that their differentiation was the result of the greater injury from the thicker knife. The complete differentiation of these fragments cut with the coarser knife may have been due to the influence of by-products from injured cells or to the actual destruction of cells at the apex of the meristem. WHITE (13) has called attention to the effect of injury on the growth of excised wheat root tips.

The results secured with excised corn root tips split longitudinally into two equal parts are similar to those observed by LOPRIORE (2, 3), SIMON (11), and NEMEC (5) on the regeneration of split roots attached to the grain or seed. SIMON found that if root tips of corn are split longitudinally for 1 cm. from the tip regeneration occurs through the activity of cells at the apex of the meristem where little or no differentiation is evident; farther back from the tip a partial regeneration takes place; and approximately 1 mm. from the end of the original root tip, only scar tissue forms over the cut surface.

Pea roots responded in the same fashion as did corn although the structure of the tip of the pea root is not the same as that of corn. Four root tips of pea were divided into equal parts by a longitudinal cut. Each of the halves developed into normal roots.

One root tip only of wheat was divided longitudinally into equal halves. The original tip was 1.5 mm. long. At the end of one week's growth each piece was 5–6 mm. long and coiled, especially at the base, with the cut surface toward the inside of the coil. The base of each fragment for 1–2 mm. was made up of large nearly cubical cortical cells and epidermal cells, 50–55 μ wide, and on one side

stelar tissue with xylem vessels had formed. Numerous root hairs were present except on the side composed of stele. Near the tip the epidermal and cortical cells were narrower and longer, 100–130 by 18 μ; the stele, still one side, did not show fully differentiated xylem vessels. The tip, surrounded by root cap cells, consisted of elongated parenchyma cells which were not embryonic. The tip was six or eight cells in width. What appeared to be the shrunken remains of dead cells were present on the inside of the coil for about 2 mm. from the base.

Evidently the cells in the half wheat root tip underwent little or no division, but differentiated, those at the tip of the meristem elongating but forming none of the specialized cells of the older portion of the root.

ROOT TIPS CUT LONGITUDINALLY INTO UNEQUAL PARTS

Eighteen root tips of corn 1–2 mm. in length were cut longitudinally with one portion larger than the other. These fragments were allowed to grow for one to three weeks. In every case the larger portion developed into a normal root with an apical meristem. All of the smaller portion differentiated. At the end of the period of growth it contained no apical meristem, and, except for a small amount of stele at the older and basal part of some of the fragments, consisted entirely of cortical and epidermal cells. A typical example of this sort of development is shown in figure 6.

When the smaller portion contained no plerome it elongated more rapidly than the larger piece, developed no root hairs, and completed its growth in two or three days. In a specific case the smaller fragment consisted of a thin slice from one side of the root tip. At the end of two days it was 8 mm. in length and no root hairs were evident. At the end of 18 days it was still 8 mm. in length and consisted of a thin filament of cortical and epidermal cells with no apical meristem and no root hairs. The larger fragment at the end of two days was 6 mm. long, slightly coiled toward the side with the plerome exposed, and had an abundance of root hairs. At the end of 18 days it was 27 mm. in length and for the major portion of its length was a normal root.

The development of the larger fragment of a corn root tip divided

longitudinally into unequal parts agrees with the results observed with fragments cut into equal parts. If each of the equal halves develops into a complete root, a fragment formed by a longitudinal cut and composed of more than half the root should develop in the same way.

The smaller fragments, in which all of the meristem differentiated, consisted of dermatogen and periblem and in some cases a small part of the plerome. Even though these fragments contained part of the plerome, they lacked the initial cells and the cells of the adjacent histogens. From an examination of prepared sections of corn root tips it was estimated that the smaller fragments lacked the tip cells of the plerome, periblem, and dermatogen for a distance of 0.15–0.2 mm. from the apex of the root meristem. The differentiation of these smaller fragments shows that those cells of the meristem which are within 0.15–0.2 mm. of the apex have totipotentialities. Cells more distant from the apex are, under the conditions of these experiments, fixed in the possibilities of their development. Further, it is evident that exposing the periblem cells does not materially affect the character of their differentiation.

The more rapid elongation of the smaller fragments which developed no stele is probably due to a greater effective internal pressure in the cells of the dermatogen and periblem than in the plerome. That such a pressure exists in the cells of the former two tissues is confirmed by the coiling of the half root, which always occurred toward the cut side. Whether this greater pressure is the result of a greater actual pressure within the cells or because of thinner and more pliable cell walls in the periblem and dermatogen, cannot be stated from these observations. The failure of the pieces composed of periblem and dermatogen to produce root hairs, in contrast to those pieces which included plerome also and which elongated more slowly but formed many root hairs, is probably explained as follows:

A piece of root which contains plerome elongates less rapidly than a piece which lacks plerome. In the latter case the pressure from within an epidermal cell against the cell wall is relieved by the stretching of the entire cell, which is possible because no plerome differentiating into stele is present to limit the stretching of the cell. Where plerome is present in the original piece, the internal pressure

in the dermatogen or the epidermis is not relieved by the stretching of the entire cell and remains sufficiently high to push out a root hair.

The results secured with pea roots were similar to those found for corn. Three root tips of pea were cut longitudinally so that one piece was noticeably larger than the other. The smaller portions contained little or no plerome. The larger fragment of each set developed into a complete root, the smaller elongated somewhat but had no apical meristem at the end of one week's development.

On the other hand, neither piece of the single wheat root tip which was cut developed into a normal root. One root tip was cut longitudinally into unequal pieces. The original tip was 1 mm. in length, and after one week the smaller fragment, A, was 4 mm. long and the larger, B, was 5 mm. long. Neither piece formed a normal root. A consisted of epidermal, cortical, and root cap cells with no stele and no root hairs. The apical meristem had differentiated into elongated parenchyma cells. B produced one branch root; stelar tissue was present but no apical meristem could be found. The stele extended almost to the tip of the root, which was composed of cortex and epidermis-like cells. Root hairs were numerous and were present over the tip of the fragment.

FRAGMENTS FORMED BY TWO OR MORE LONGITUDINAL CUTS

Fourteen excised corn root tips 1–2 mm. in length were divided into three or more fragments by longitudinal cuts. In four sets of such fragments no terminal meristem was present after one week of development. All of the apical meristem had differentiated. In all others the largest fragment grew and formed a normal cylindrical root with a terminal meristem. Even though strips of periblem and dermatogen were cut from as many as three sides of the tip, if half or more than half of the plerome remained the piece developed into a normal cylindrical root. When the root tip was divided longitudinally less than half of it was sufficient in these experiments for the production of a normal root, but a quarter of the root tip did not form a normal root.

Two examples are illustrated in figures 7 and 8. In figure 7 the results are shown for a root tip which was divided longitudinally into two unequal parts. The larger part was divided by a longitudinal

cut perpendicular to the cut surface into two equal parts, B and C. After one week the smaller fragment, A, of the original tip was 6 mm. long and completely differentiated. B and C were each 12 mm. long and had developed into normal roots.

In the experiment illustrated in figure 8 the original root tip was divided longitudinally into two equal halves and one of the halves was cut again into equal parts, B and C. A represented half the root tip, B and C, one quarter of it. After one week A was 21 mm. long and had formed a complete root. B and C were each 4 mm. long and both had differentiated completely. It is interesting to note that even in the quarter root tips the pericycle functioned normally and formed branch roots.

Two root tips of pea were each cut into three fragments by longitudinal cuts. The results were similar to those secured with corn. A piece which was less than half but more than one quarter of the root developed into a normal root tip. A root tip 2.2 mm. in length was divided unequally by a longitudinal cut into piece A and a larger piece. The larger piece was divided equally into B and C. At the end of one week B and C each possessed normal root tips while A lacked one.

From the experiments on the growth of fragments of root tips formed by longitudinal cuts, it is evident that all parts of the apical meristem are not equally important in determining the tissues which are differentiated and the persistence of an embryonic region.

Pieces which did not include the apex of the plerome did not form normal roots even though they included meristem from the dermatogen and periblem to within an estimated 0.15 or 0.2 mm. of the apex of the meristem. While this statement emphasizes the importance of the apex cells of the plerome for the differentiation of a normal root and for the persistence of the apical embryonic region, the apex cells of the dermatogen and periblem may be equally important since they also were included in the fragments which developed normally. The initial cells, however, are not necessary for normal development.

Quantitative relations also are important since normal development did not occur even if apex cells of the plerome were included in
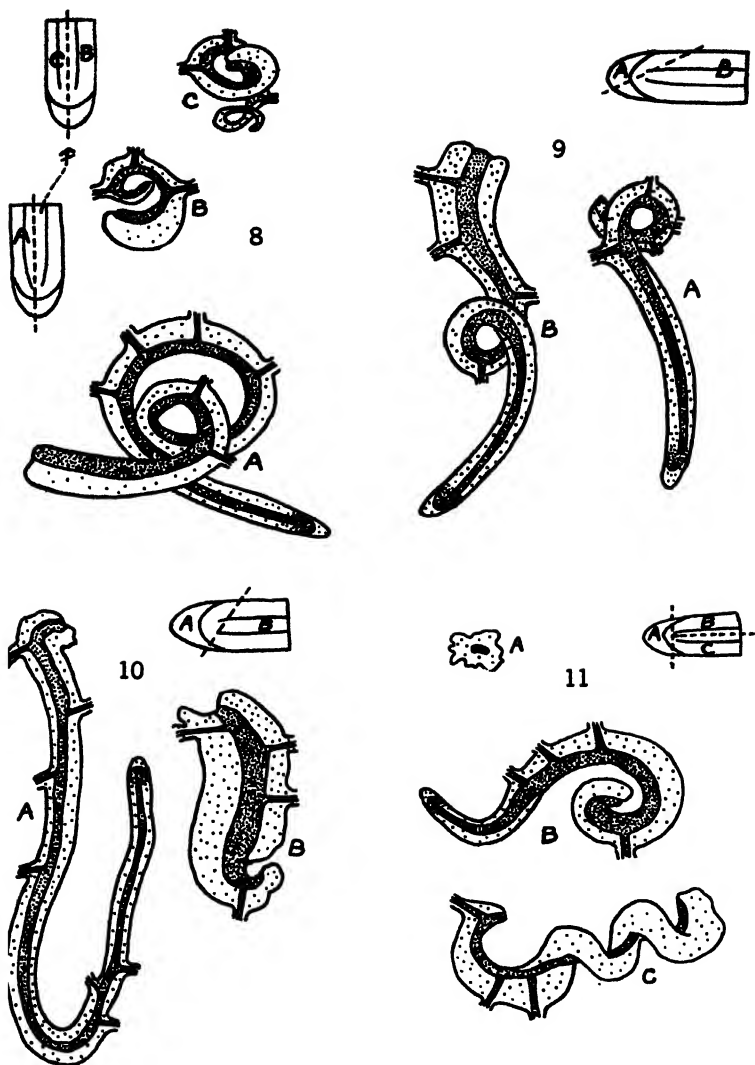
FIGS. 8–11.—Fig. 8, development of fragments of corn root tip originally 2.0 mm long and cut longitudinally into equal parts, one of these then cut longitudinally into equal parts. Half fragment developed into a normal root; quarter fragments lacked root cap and apical meristem. Fig. 9, development of fragments of corn root tip originally 2.0 mm. long and cut obliquely through apex of meristem; the terminal fragment measured 0.7 mm. in length. Both pieces formed normal roots. Fig. 10, development of fragments of corn root tip originally 1.3 mm. long and cut obliquely back of apex of meristem. Terminal piece formed a normal root; basal piece differentiated but lacked root cap and apical meristem. Fig. 11, development of fragments of corn root tip originally 1.5 mm. long and cut as shown in diagram. Terminal piece was 0.5 mm. long and formed an unorganized mass; the larger piece, B, developed a normal root and the smaller piece, C, lacked root cap and apical meristem.

the fragment, unless (in corn and pea) more than one fourth of the group was present.

Furthermore the treatment of the fragment is concerned, as is illustrated by the failure of corn root tips to develop into normal roots if they were cut longitudinally with a thick razor blade. In this case the significant fact is that the group of cells which normally remains embryonic lost its embryonic character and differentiated.

The number of wheat root tips cut longitudinally is too small to permit dogmatic conclusions. Nevertheless the results, which contrast with those secured with corn and pea, are of interest. The smaller size of the wheat root tips and the smaller quantity of meristem in a longitudinal section of a tip may suggest the explanation for the failure of such fragments to form complete roots. A second possibility is that the longitudinal cut destroyed certain apical meristematic cells necessary for the continued development of meristem.

FRAGMENTS FORMED BY OBLIQUE CUTS

Twelve root tips of corn were cut obliquely, with the cut dividing the embryonic region into two parts. In four of the tips the cut was made as nearly as possible through the apex of the meristem. Both fragments from two of these tips developed into normal roots. The results in one case are illustrated in figure 9. The original root tip was 2 mm. in length. The smaller fragment, A, measured approximately 0.7 mm. on the longer side. After one week A was 15 mm. in length, B was 20 mm., and the terminal portion of each was a normal root with apical meristem.

The fragments of the other two root tips cut in this manner elongated somewhat. The tip portions formed thin filaments of parenchyma cells. The basal portions produced root hairs and branch roots. No apical meristem was present.

Eight of the root tips were cut obliquely through the apical meristem but back of its apex 0.5–0.75 mm. from the end of the root and more nearly transverse. Without exception the tip fragment in each set developed into a normal root and the basal fragment differentiated but lacked an apical meristem at the end of the experiment. One such experiment is illustrated in figure 10. After 15 days the tip portion, A, was 30 mm. long and a normal root. The basal portion,

B, was 8 mm. long, and while it had normal branch roots, no apical meristem was present.

One root tip of corn was cut diagonally through the basal portion back of the apical meristem. The tip fragment, originally 1.9 mm. on the longer side, developed into a normal root. The rear fragment in addition to numerous branch roots formed a slender normal root which apparently developed from the cut surface. It is probable that true regeneration was not involved in this case but that the development of a branch root occurred as described by SIMON (11) for decapitated roots attached to the grain.

When oblique cuts were made through the apex of the meristem, both pieces of a set of fragments formed complete roots in two cases and neither piece formed complete roots in two additional cases. Why should this be? We cannot define the exact line of the cut when oblique cuts were made through the apex of the meristem nor say exactly what cells were included in each of the two fragments formed. However, when both pieces formed complete roots sufficient meristem must have been included in the terminal piece for normal development. Judging from the preliminary experiments in which most of the fragments were removed by transverse cuts, the terminal piece probably included cells for a distance of 0.1 mm. from the apex of the meristem. This estimate is supported by diagramming a root tip and noting where an oblique cut through the apex might run. When neither piece developed a complete root the cut may have been more nearly through the apex of the meristem, forming a terminal piece with too little meristem to permit development. In this case, however, there is no obvious reason why the basal portion should not develop normally. It is possible that failure was due to injury from the cut, as appeared to be the case in the three sets of fragments cut longitudinally with a Gitsnife.

One pea root tip 2 mm. in length was cut diagonally through the meristem. Each piece at the end of one week had produced a normal root.

Five root tips of wheat were cut diagonally through the apex of the meristem. None of these fragments developed into normal roots. The tip fragments at the end of the period of growth consisted of masses of root cap cells and filaments of parenchyma cells. The bas-

al portions elongated somewhat and differentiated stele, cortex, and epidermis with root hairs but formed no apical meristem. An occasional branch root was formed by the basal pieces.

Nine root tips of wheat approximately 2 mm. in length were cut diagonally through the basal portion of the apical meristem, the tip fragments measuring 0.7–1.1 mm. on the longer side. All the tip fragments except one continued development and formed normal roots. The one exception was torn in cutting. The basal fragments, except the one which was torn, produced thin normal roots which developed from the plerome and initiated development a short distance back of the cut surface.

The regeneration of the apical meristem and root cap by the basal fragments of wheat root tips cut diagonally just back of the apical meristem is similar to that described for decapitated roots of corn and other plants by SIMON (11) and NEMEC (5).

FRAGMENTS FORMED BY TRANSVERSE CUTS

In addition to the preliminary experiments in which corn root tips were fragmented by transverse cuts, a few observations were made on wheat.

Two root tips of wheat were cut transversely through the embryonic region. The tip fragments measured 0.3 and 0.45 mm. respectively; the basal fragments were 2 and 1.45 mm. in length. None of these pieces developed into normal roots. After one week the tip portions were masses of root cap and parenchyma cells, with some of the parenchyma cells organized as filaments. The basal fragments differentiated a stele and produced branch roots but had no apical meristem. These results are similar to those in which oblique cuts were made through the apex of the meristem. WHITE (13) secured development of wheat root tips (with root cap removed) less than 0.1 mm. in length.

Three root tips of wheat were cut transversely through the base of the apical meristem, the tip fragments measuring about 0.75 mm. in length. Two of the tip fragments formed normal roots; one formed a mass of cells. The basal fragments of two of the root tips elongated to twice their original length and formed stele but developed no apical meristem. The basal piece of one of the sets of fragments in

which the tip portion grew normally showed at the end of two days the beginning of the regeneration of an apical meristem.

FRAGMENTS FORMED BY COMBINATION OF LONGITUDINAL, TRANSVERSE, AND OBLIQUE CUTS

In addition to the experiments described, a few root tips were fragmented into three or more pieces by various types of cuts.

A corn root tip (fig. 11) was cut transversely so as to include the very tip of the embryonic region in the terminal piece, A. The basal portion was then divided longitudinally into two unequal parts; a larger, B, and a smaller, C. The tip fragment showed little development and after a week consisted of a small gelatinous mound with a central core of cells surrounded by loose root cap cells. B developed into a normal root and C increased in length and formed branch roots but had no apical meristem. B and C each measured 7 mm. at the end of one week.

From another tip (fig. 12) two side portions, C and D, were removed and the remaining portion was divided into two pieces by a transverse cut well up in the embryonic region. The tip portion, A, contained part of the root cap and the apex of the meristem, the rear portion, B, contained part of the meristem but not its apex. The side fragments, C and D, which were cut so as to avoid including any of the plerome, elongated from 1.1 to 12 and 14 mm. in seven days, and consisted of a filament of cortical and epidermal cells. No apical meristem was present. The tip fragment, A, elongated from approximately 0.6 to 9 mm. and formed an entirely normal root. The basal fragment, B, differentiated a stele and formed branch roots but no apical meristem.

In figure 13 the development of a central piece of a corn root tip is illustrated. The original root tip was approximately 1.4 mm. in length. A transverse cut was made just back of the attachment of the root cap. Longitudinal cuts were made on two sides of the remainder of the root tip. These cuts were designed to include a small amount of the plerome. The central piece only was grown. After one week the resulting structure was 11 mm. long and had formed a normal tip with apical meristem as well as branch roots.

Two root tips were cut as shown in figure 14. In each the tip frag-
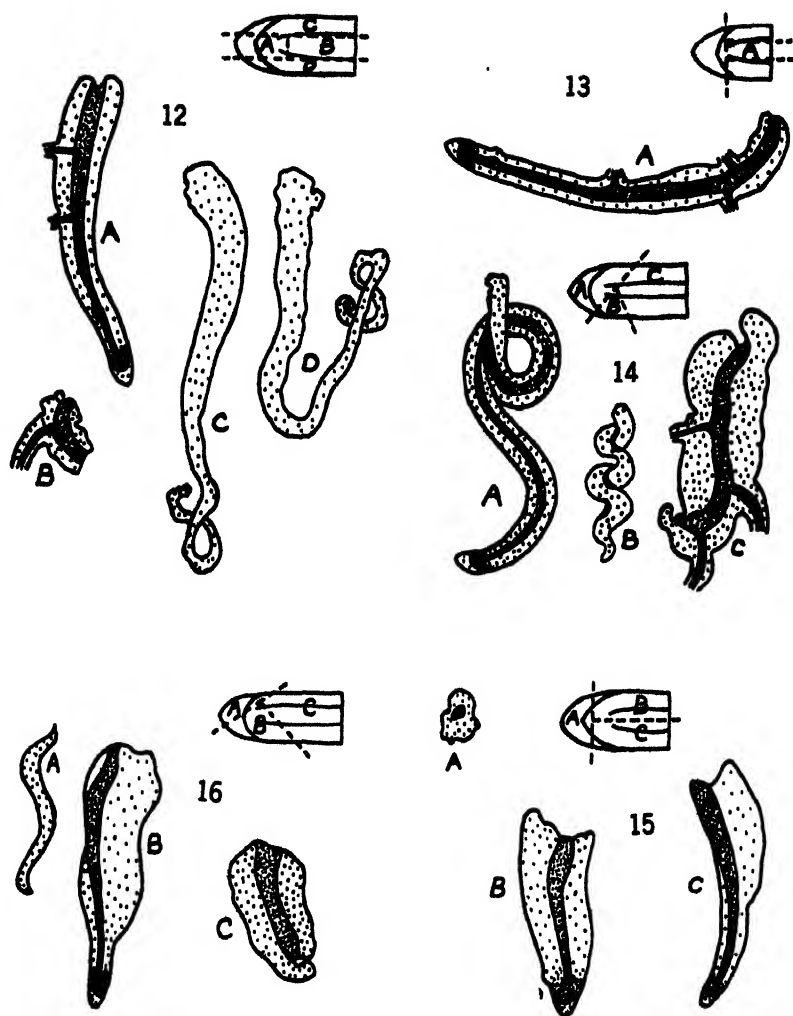
FIGS. 12–16.—Fig. 12, development of fragments of corn root tip originally 1.1 mm. long and cut as shown in diagram. Fig. 13, development of central fragment (0.8 mm. long) of corn root tip cut as indicated in diagram. Fig. 14, development of fragments of corn root tip originally 1.5 mm. long and cut as shown in diagram. Terminal piece, *A*, developed into a normal root; *B* and *C* lacked root cap and apical meristem. Fig. 15, development of fragments of pea root tip originally 2.5 mm. long and cut as shown in diagram. Terminal piece 0.5 mm. long; basal piece cut into equal parts. Fig. 16, development of fragments of pea root tip originally 1.8 mm. long and cut as shown in diagram. *A* developed into filament of cortical and epidermal cells; *B* formed normal root; *C* lacked root cap and apical meristem.

ment, A, formed a normal cylindrical root with an apical meristem. The small wedge-shaped fragments, B, just back of the tip elongated from their original lengths of 0.5 and 0.8 mm. to 5 and 8 mm. respectively. The fragments, C, elongated from 1 and 1.5 mm. to 6 and 8 mm. respectively and formed numerous branch roots. The cells in C were large, 150 μ by 105 μ as compared with normal cells of 42 μ by 18 μ. It is particularly interesting that normal polarity is shown by the wedge shaped pieces. Although they did not develop into normal roots, the cells of which they were originally composed elongated in the same direction as they would have in the normal root, producing a filament and not an unorganized mass.

A pea root tip 2.5 mm. long was fragmented as shown in figure 15. The tip fragments, approximately 0.5 mm. long, formed a mound of cells. B and C each developed into normal roots measuring at the end of one week 6 and 4 mm. respectively.

One root tip of pea originally 2.4 mm. in length was fragmented as shown in figure 16. The wedge shaped piece, B, which included the major portion of the apical meristem, formed a complete root tip. The other two pieces, A and C, did not.

Discussion

In general the results of these experiments confirm the experiments of PRANTL (6), SIMON (11), LOPRIORE (2, 3), and NEMEC (5) on the regeneration of decapitated or split roots. In their experiments the decapitated or split roots were attached to the grain while in the experiments described in this paper excised root tips cultivated in pure culture were used.

Although accurate comparison of the results secured with excised and with attached root tips cannot be made, it would appear that the regenerative power of decapitated excised corn root tips under the conditions of our experiments is less than that of roots attached to the grain. Regeneration of a new root tip by the excised corn roots was observed only when decapitation was near the apex of the root tip. None of the basal pieces from fragments formed by cuts farther back in the meristem regenerated, although in SIMON's experiments with attached roots they did. Excised wheat roots appear to have greater powers of regeneration than do those of corn, as re-

generation was observed in the majority of cases in which the excised root tip was decapitated back in the meristem some distance from the apex. The experiments with peas were too few to permit any conclusion other than that regeneration may occur.

SIMON, by anatomical studies and by experiments in which specific tissues of the root were removed and destroyed, concluded that the pericycle is primarily concerned in regeneration by decapitated roots. NEMEC, who studied regeneration in roots wounded in the meristem by cuts of various depths and directions, concluded that the interruption by the cut of "correlations" between the apex of the meristem and the remainder of the root was the chief cause of regeneration. The pericycle appeared to be the tissue through which the correlation was effective, as regeneration did not occur unless half or more than half of the pericycle was severed in wounding.

In our experiments the origin of regeneration from the plerome in decapitated excised wheat root tips and the failure of strips of the periblem to regenerate an apical meristem are in accord with the view that the pericycle is of prime importance in the regeneration of a new apical meristem and root cap by decapitated roots.

The results secured with excised root tips split longitudinally are similar to those found by SIMON and LOPRIORE for split roots attached to the plant. Evidently a quantitative relation is involved here. One quarter of an excised corn root tip divided longitudinally did not regenerate a new root tip, although a piece amounting to between one quarter and one half of the root tip did. This is of interest since NEMEC found that regeneration did not occur in the attached roots he used unless more than one half of the pericycle was severed.

These experiments, however, are exploratory rather than definitive. They show that a decapitated root tip 1 mm. or so in length contains within itself all the essentials for regenerating a new apical meristem and root cap if it is grown on an agar medium containing mineral salts and glucose. They suggest that the cultivation of excised root tips may permit a more complete analysis of the physiology of regeneration than is possible when the root tips are attached to the plant and supplied with all the materials which may be derived therefrom.

In addition to the observations made on the regeneration of de-capitated excised root tips, the writers' experiments include some on the development of fragments of the apical meristem. Four types of development of isolated bits of the apex of a root tip were observed: (a) little or no development; (b) formation of filaments of parenchyma cells; (c) formation of a root in which stelar tissue differentiated but no apical meristem persisted; (d) formation of a normal root. The experiments do not permit such desirable analysis of these different types of development as, for example, to determine how far they depend upon the kind of tissue included, upon the quantity of tissue included, and upon the treatment (injury from cutting, nutrient conditions) of the isolated fragment. They suggest that if normal development is to occur in the fragment there must be included a portion of the plerome at the apex of the meristem between 0.1 and 0.2 mm. in length and equivalent to between one quarter and one half of that found at the root apex. Fragments of the root tip apex which do not contain this amount of the apical plerome fail to develop normally. The initial cells at the apex of the meristem need not be included in the pieces used. Here again the experiments are largely exploratory. Sufficient data have been secured, however, to show that the methods used may permit a more complete analysis of the factors involved in the existence of meristematic tissue in root tips and its differentiation.

Summary

1. Excised root tips of corn cultivated under sterile conditions on a nutrient medium grew in the majority of cases if they were 0.5 mm. or more in length, and in all cases if they were 0.9 mm. or more in length.

2. When excised root tips of corn and of pea 1-2 mm. long were divided longitudinally into equal parts, both pieces developed into complete roots on the nutrient medium used.

3. When excised root tips of corn and of pea 1-2 mm. long were divided longitudinally so that one part contained most of the plerome, the larger part developed on the media used into complete roots; the smaller grew but lacked one or more parts of a complete root.

4. When excised root tips of corn and of pea 1–2 mm. long were divided longitudinally into three or more pieces, a piece comprising less than one half of the root developed into a complete root; but one quarter, under the conditions of the experiment, did not.

5. When excised root tips of corn 1–2 mm. long were cut obliquely through the apex of the meristem, both pieces in some cases developed into complete roots; in others the terminal piece formed a filament of cells and the basal piece differentiated but possessed no apical meristem at the termination of the experiment.

6. When excised root tips of corn 1–2 mm. long were cut obliquely through the basal portion of the meristem, the tip fragment developed into a complete root but the basal fragment differentiated and formed no apical meristem.

7. When excised root tips of wheat 1–2 mm. long were cut longitudinally, neither piece developed into a complete root; both lacked an apical meristem at the completion of the experiment.

8. When excised root tips of wheat 1–2 mm. long were cut obliquely or transversely through the apex of the meristem, neither piece formed a complete root. The tip fragment formed masses of root cap cells and filaments of parenchyma cells; the basal portions elongated and differentiated but formed no apical meristem.

9. When excised root tips of wheat were cut obliquely or transversely through the basal portion of the apical meristem, the tip fragments developed into complete roots and the basal fragments regenerated a new apical meristem.

10. Four types of response of fragments of the apical meristem of root tips were observed as follows: (a) Little or no development; (b) formation of filaments of parenchyma cells; (c) formation of a root in which stelar tissue differentiated but no apical meristem remained; (d) formation of a normal root.

11. Injury from the cut may affect the character of the development of fragments of the apical meristem of roots.

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LITERATURE CITED

1. Chambers, W. H., Culture of plant cells. Proc. Soc. Exp. Biol. and Med. 21:71–72. 1923.

2. Lopriore, G., Ueber die Regeneration gespaltener Wurzeln. Nov. Act. Leop. Carol. Deut. Akad. Naturf. 66:211–286. 1896.

3. ———, Regeneration von Wurzeln und Stämmen infolge traumatischer Einwirkungen. Inter. Bot. Kong. 1905:242–278. 1906.

4. Maneval, W. E., Lactophenol preparations. Stain Technol. 2:9–11. 1936.

5. Nemec, B., Studien über Regeneration. p. 387. Berlin. 1905.

6. Prantl, K., Regeneration des Vegetations punktes an den Angiospermen- wurzeln. Arb. Bot. Inst. Würzburg 1:546. 1874.

7. Robbins, W. J., Cultivation of excised root tips and stem tips under sterile conditions. Bot. Gaz. 73:376–390. 1922.

8. ———, Effect of autolized yeast and peptone on growth of excised corn root tips in the dark. Bot. Gaz. 74:59–79. 1922.

9. ———, and Maneval, W. E., Further experiments on growth of excised root tips under sterile conditions. Bot. Gaz. 76:274–287. 1923.

10. ———, and Maneval, W. E., Effect of light on growth of excised root tips under sterile conditions. Bot. Gaz. 78:424–432. 1924.

11. Simon, S., Untersuchungen über die Regeneration der Wurzelspitze. Jahrb. Wiss. Bot. 40:103–143. 1904.

12. Strasburger, E., Textbook of botany. 6th English ed. p. 1261. 1930.

13. White, P. R., Plant tissue cultures. A preliminary report of results ob- tained in culturing of certain plant meristems. Archiv. Expt. Zellf. 12:602– 620. 1932.

ADDITIONS TO THE GENUS EUPHORBIA L. AND TO CERTAIN GENERA OF THE COMPOSITAE

EARL EDWARD SHERFF

Euphorbia olowaluana sp. nov.—Frutex forsitan arborescens, ramosus; ramis brunneo-griseis (ultimis herbaceis saepius tenuissimis moniliformibus glabratis vel minutissime hispidulis, nodulis perspicuis). Folia disticha saepius squarrosa; petiolo tenui aegre puberulo 1–2 mm. longo; lamina oblonge lineari, apice obtusa vel acuta, basi inaequilaterali (sed tantum circ. 1–2 mm. lata), membranacea, glabrata, subtus venis obliquis brunneo-purpureis ornata, 1.5–2.5 (raro –3) cm. longa et 4–8 mm. lata; 2 stipulis in corpus interpetiolare primum triangulatum coalitis. Capitula plerumque solitaria in axillis etiam ad ramulorum apices. Involucrum subsessile campanulatum urceolatumve extus glabratum vel summam versus tomentellum, circ. 2.3 mm. altum, glandulis 4 transverse oblongis subcontiguis. Capsula cernua, profunde trigona, glabra, brunnea, circ. 2.3 mm. alta, coccis aegre carinatis carina atriore; stylis basi connatis, breviter bifurcatis ramis valde incrassatis; stipite pubescenti manifesto. Semina ovata, tetragona, brunnea (primum raro subgrisea), scrobiculata, 1.3–1.5 mm. longa.

Specimens examined: *Charles N. Forbes 2242M*, Olowalu Valley, Isl. Maui, May 7, 1920 (Herb. Field Mus.); *idem 2341M*, central ridge of Olowalu Valley, Isl. Maui, May 12, 1920 (type, Herb. Bishop Mus.).

Euphorbia olowaluana and its var. *gracilis* (*vide infra*) appear to constitute a species intermediate between *E. multiformis* H. & A. (as to often acutish leaves) and its var. *microphylla* Boiss. (as to herbaceous moniliform ultimate branchlets) on the one hand and its var. *manoana* and *E. celastroides* vars. *amplectens* and *mauiensis* (as to appearance of leaves, especially of the brownish purple venation) on the other.

In view of the existence of the many varieties of *E. multiformis* and

E. celastroides, the recognition here of *E. olowaluana* and the var. *gracilis* as apart from them must needs be somewhat arbitrary.

EUPHORBIA OLOWALUANA var. **gracilis** (Rock) comb. nov.; *E. lorifolia* var. *gracilis* Rock Indig. Trees Haw. Isls. 259, pl. 100. 1913.

EUPHORBIA HILLEBRANDII **palikeana** var. nov. Degener & Sherff.— Inflorescentia 3–10-cephala valde contracta 0.5–1.5 cm. longa nodulis bracteolatis numerosis perspicuisque.

Specimens examined: *Otto Degener, Kwan Park,* and *Will Bush* 8049, open woods, in third small valley northeast of Palikea, Isl. Oahu, Sept. 19, 1932 (type, Herb. N.Y. Bot. Gard.: cotype, Herb. Degener).

MR. OTTO DEGENER, the well-known authority on the flora of the Hawaiian Islands, courteously permits the use of his name, although somewhat provisionally, in joint authorship for this and certain other novelties (*vide infra*) collected by himself and his associates and described in this paper. He himself had been inclined to regard the indigenous Hawaiian Island species as deserving generic segregation from *Euphorbia,* a view which my own studies do not permit me to share.

EUPHORBIA HILLEBRANDII **wainianana** var. nov.—Rami adscendentes, virgati, sulculati, glabri, moderate nodosi, internodiis saepius 1.3–3 cm. longis. Foliorum petioli tenues patenti-hispiduli, 1–2 mm. longi; laminae elliptico-lanceolatae vel (saepe subrhomboide) oblanceolato-oblongae, apice subacutae vel acutae et fere mucronatae, basi inaequilaterali angustatae vix subtruncatae, glabrae, membranaceae, margine vix subrevolutae, infra paulo pallidiores, 2–4 cm. longae et 7–15 mm. latae; corporibus interpetiolaribus lato-triangularibus ∓0.5 mm. altis. Capitula axillaria terminaliaque (interdum ad ramulos minutos subcapilliformes axillares), solitaria vel subsolitaria. Involucrum campanulatum, minutum (sub 1.5 mm. altum), extus glabratum; lobis hirtis; glandulis 5, transverse oblongis, plus minusve contiguis, exappendiculatis; floribus masculis exsertis; pedicella glabra sub 1 mm. longa. Capsula submatura glabra, vix 2 mm. alta, coccis moderate carinatis, non sulcatis; stipite glabro, ∓4 mm. longo; stylis distinctis, fere usque ad medium bifurcatis, seminibus non visis.

Specimens examined: *Charles N. Forbes* (with *Dean Lake*) 1978*O*, Wainiana Ridge, Isl. Oahu, Oct. 27–30, 1914 (type, Herb. Bishop Mus.).

Euphorbia halemanui sp. nov.—Glabra, verisimiliter frutex 1–2 m. altus, ramorum internodiis plerumque 0.5–1.5 cm. longis. Folia opposita, tenuiter petiolata petiolis 5–10 mm. longis; lamina elliptice vel interdum subrhomboide oblonga vel obovata, apice obtusa nec emarginata, basi angustata sed vix inaeqilaterali, marginibus non vel parce subrevoluta, penninervi nervis divaricatis supra saepe obscuris, 4.5–8 cm. longa et 2–3.3 cm. lata; corpore stipulari interpetiolari, triangulari, scarioso, margine superiore fimbriato, 1–2 mm. alto. Inflorescentia contracta subglobosa (tantum 4–8 mm. alta), axillaris terminalisque, circ. 4–10 capitulis ad utrumque nodum. Involucrum urceolatum, extus glabrum, sub 2 mm. altum; glandulis 4 vel 5, transverse oblongis, exappendiculatis; pedicella minuta, glabra, sub 3 mm. longa; floribus masculis numerosis exsertis. Capsula submatura cernua stipite glabro sub 3 mm. longo, glaberrima, trigona, 2.2 mm. alta, coccis subacriter carinatis; stylis usque ad basim valde distinctis, lobis brevibus incrassatis.

Specimens examined: *Charles N. Forbes* 943*K*, Halemanu, Isl. Kauai, Jul. 3–Aug. 18, 1917 (Herb. Bishop Mus.); *idem* 1095(*a*)*K*, same locality and date (type, Herb. Bishop Mus.); *Dr. Carl Skottsberg* 1002, forest near Kokee Ranger Station, Isl. Kauai, Oct. 28, 1922 (Herb. Bishop Mus.).

Noticeable for its minute subglobose very much contracted clusters of heads, a cluster usually measuring 4–7 mm. tall.

Euphorbia forbesii sp. nov.—Glabra, probabiliter frutex plus minusve erectus; caulis internodiis saepius 1.5–3.5 cm. longis, siccis brunneo-rubris. Folia opposita, non numerosa, vix petiolata petiolis tantum circ. 1–3 mm. longis; lamina oblonge ellipticeve oblanceolata, apice obtusa et saepe paulo emarginata, basi inaequilaterali (unico latere saepe subcordata), crassiuscula vel membranacea, leviter revoluta, infra pallidiore, penninervi nervis lateralibus numerosis obscuris divaricatis, foliorum principalium 10–16.5 cm. longa et 2.5–4 cm. lata: stipulis interpetiolaribus late triangularibus superne angustatis omnino circ. 2 mm. altis. Inflorescentiae cymae axillares terminalesque, apertae, plus minusve valde decompositae ramis

ramulisque non valde patentibus, usque ad 7.5 cm. longae, 8–15-
cephalae. Involucrum hemisphaericum, extus plus minusve pubes-
cens, 1.5–3.5 mm. altum; lobis ovatis, hispidis; glandulis 4 vel saepius
5, latissime stipitatis, transverse oblongis (perspicue reniformibus),
exappendiculatis, siccis purpureo-nigris; pedicella tenui, angulata,
sulcata, glabrata vel saepius minute patenti-hispidula, saepius 1–2.2
cm. longa. Capsula submatura angulato-cylindrica, utrinque trun-
cata, glabra, circ. 6 mm. alta, cernua, stipite glabrato circ. 3–4 mm.
longo; stylis distinctis, ramis non valde separatis; seminibus non visis.

   Specimens examined: *Charles N. Forbes* 1770O, Makuleia, slopes
of Puu Kaala, Isl. Oahu, Apr. 26–May 16, 1912 (Herb. Bishop Mus.;
Herb. Field Mus.); *idem* 2218O, Wahiawa ditch trail, Isl. Oahu,
Aug. 17–20, 1915 (type, Herb. Field Mus.: cotypes, Herb. Kew;
Herb. Missouri Bot. Gard.); *Joseph F. Rock* 3037, Kaukonahua,
Wahiawa, Isl. Oahu, May 15, 1909 (Herb. Gray; Herb. N.Y. Bot.
Gard.); *Harold St. John* 10629, shrubs 8 ft. tall, alt. 1800 ft., shady
woods on ridge, South Opaeula Gulch, Paalaa, Koolau Mountains,
Isl. Oahu, Nov. 9, 1930 (Herb. Berl.; Herb. Delessert; Herb. Kew).

   Euphorbia clusiaefolia var. *grandifolia* Hillebr. (Fl. Haw. Isls. 395.
1888) was described by HILLEBRAND evidently from the lone sterile
branch preserved in his herbarium (Herb. Berl.; the specimen is now
before me). This branch came from "Makaleha in the Kaala range"
and doubtless belongs to *E. forbesii* proper or else to its closest rela-
tive, *E. rockii* Forbes. Both of these species are found near Puu
Kaala.

   Euphorbia degeneri nom. nov.; *Euphorbia cordata* Meyen Bei-
träge Bot. Reise Erde 2:150. 1843 (*non* Räuschel *nec* Schrank).—
Named for MR. OTTO DEGENER, who independently had noted (*in
herb.*) the desirability of a new name for this species to avoid confu-
sion with *E. cordata* Schrank.

   EUPHORBIA DEGENERI **molokaiensis** var. nov.—Folia capsulaeque
velutino-pilosae.

   Specimens examined: *Otto Degener* 8067 *pro parte*, along beach in
arid region, near Waiakanapo, Isl. Molokai, Apr. 19, 1928 (Herb.
Degener; Herb. Field Mus.); *Joseph F. Rock* 7078 *pro parte*, Moom-
omi, Isl. Molokai, March, 1910 (type, Herb. Gray: cotype frag-
ment, Herb. Field Mus.).

EUPHORBIA REMYI **waimeana** var. nov.—Folia moderate magis coriacea revolutaque, saepius obovato-oblonga, basi saepius sub-angustata et inaequilateralia, lamina plerumque circ. 7–8.5 cm. longa et 4–5 cm. lata. Unicum caput sine fructu visum.

Specimens examined: *Charles N. Forbes* 1043 *K*, Kalalau-pali, Waimea Drainage Basin, west side, Isl. Kauai, Jul. 3–Aug. 18, 1917 (type, Herb. Field Mus.: cotypes, Herb. Berl.; Herb. Delessert; Herb. Kew).

Confusion has arisen in literature owing to HORACE MANN's publishing in 1867 (Proc. Amer. Acad. 7:201; Enum. Haw. Pl. no. 438) what purported to be an original description of *E. remyi* A. Gray, with Kauai cited first (*i.e.*, before Oahu) for the habitat. We must note, however, that some five years previously BOISSIER (*in* DC. Prodr. 15$^{\text{II}}$:1262. 1862) had published *E. remyi* A. Gray and cited Oahu first. In fact, BOISSIER listed two varieties, *a* and *β*, and based the var. *a* directly upon *Remy* 598 from Oahu.

I have found no other specimens matching the *Remy* type (now before me), and it may well be that the species proper is one of those probably numerous endemic Hawaiian species which have been exterminated within the past century. There are, however, various specimens from Kauai that fall into eleven more or less easily distinguishable varieties of *E. remyi*, one of these being the var. *waimeana*. This variety may be the first of the two forms described by HILLEBRAND (Fl. Haw. Isls. 395. 1888) as having been collected by *Knudsen* in Waimea. HILLEBRAND described the stipules as low, triangular, and 1–1.5 lines long. This holds for the very few stipules still observable toward tips of branches on my type, but the stipules lower down are represented merely by the whitish interpetiolar callosities which are conspicuous in this species.

EUPHORBIA ṚEMYI **kauaiensis** Degener & Sherff var. nov.— Foliorum petioli tenues, 5–18 mm. longi; lamina elliptico-oblonga vel rarius -lanceolata vel subrhomboideo-oblonga, apice subacuta rarissime emarginata, basi plus minusve inaequilaterali cuneato-angustata vel raro subrotundata, subnitida, membranacea, 8–15.5 cm. longa et 3–5 cm. lata, penninervi nervis lateralibus divaricatis tenuibus manifestis, integerrima, non vel minutissime irregulariterque revoluta. Inflorescentia axillaris terminalisque, irregulariter

ramosa nodulis stipuliferis saepe 2–gemmatis, 1.5–4 cm. longa, plerumque 3–7-cephala. Involucrum siccum atrum vel purpureo-atrum, hemisphaericum, extus glabratum vel supra hispidulum, 3 mm. altum; lobis parvis, hirtis; glandulis plerumque 5, transverse oblongis, exappendiculatis; pedicella tenui, atra, glabrata vel spar-sim setosa (saepe irregulariter minutissimeque plus minusve tenui-papillata), saepius 7–10 mm. longa. Capitula cylindrico-ovata, suba-criter trigona, glabra, breviter stipitata (stipite semper recto vel subrecto videtur), 3.5–4 mm. alta; stylis usque ad basim distinctis, lobis brevibus vel brevissimis incrassatis. Semina castanea, acriter tetragona, basi truncata apice obtusa, faciebus rugoso-scrobiculata, circ. 2–2.2 mm. longa.

Specimens examined: *Otto Degener* 8093, near Hanapepe Falls, Isl. Kauai, June 19, 1926 (type, Herb. N.Y. Bot. Gard : cotypes, Herb. Degener, two sheets; Herb. Field Mus.).

EUPHORBIA REMYI **pteropoda** var. nov.— Folia robuste petiolata petiolis latis marginatis 6–12 mm. longis; lamina late elliptico-ob-longa, interdum minute emarginata, moderate revoluta, subcoriacea, 8–11 cm. longa et 4–4.8 cm. lata.

Specimens examined: *Abbé Urbain Faurie* 478, alt. 800 m., Kauhao, Isl. Kauai, February, 1910 (type, Herb. Delessert).

A variety quite distinct from the species proper and the other varieties in its robust, margined leaf petioles and wide leaf rachises. Only in the var. *olokelensis* (*vide infra*) do the leaf petioles or rachises tend at all to simulate those of var. *pteropoda* and then only oc-casionally and only to a slight extent.

EUPHORBIA REMYI **lydgatei** var. nov.—Folia tenuiter petiolata petiolis 7–10 mm. longis; lamina oblongo-obovata, apice ipso abrupte subacuta, basi angustata inaequilateralique, aegre vel parce revoluta, membranacea, 11–15.5 cm. longa et 5–7 cm. lata, nervis mani-festis.

Specimens examined: *Reverend J. M. Lydgate*, Pole Line Trail, Wailua Mountains, Isl. Kauai (type, Herb. Bishop Mus.).

EUPHORBIA REMYI **leptopoda** var. nov.—Folia tenuiter petiolata petiolis obsolete hispidis 8–22 mm. longis; lamina saepius elliptico-oblonga (raro ovato-oblonga vel subrhomboideo-oblonga), apice ob-tuso interdum minute emarginata, basi rotundata vel subcordata

et saepius inaequilaterali, non vel tenuissime subrevoluta, nitida, saepius 9–13 cm. longa et 3–4.5 (–5.3) cm. lata.

Specimens examined: *Charles N. Forbes* 1095K, Halemanu, Isl. Kauai, Jul. 3–Aug. 18, 1917 (type, Herb. Bishop Mus.).

EUPHORBIA REMYI **kalihiana** var. nov.—Folia tenuiter petiolata petiolis 3–6 mm. longis; lamina subrhomboideo-oblonga, apice plerumque moderate acuminata, basi saepe inaequilaterali cuneato-rotundata, membranacea, leviter revoluta, tantum 4–7 (rarius –8.5) cm. longa et 2–3.5 cm. lata, venis supra obscuris.

Specimens examined: *Charles N. Forbes* 10K, at foot of Kalihi, Isl. Kauai, Jul. 8, 1909 (type, Herb. Field Mus., 3 sheets).

EUPHORBIA REMYI **wahiawana** var. nov.—Folia tenuiter petiolata petiolis 5–15 mm. longis; lamina nunc anguste nunc late oblongo-obovata, ad basim plus minusve inaequilateralem saepe sensim angustata, apice subacuminata, membranacea, leviter revoluta, 7–14 cm. longa et 3–5 cm. lata.

Specimens examined: *Charles N. Forbes* 177K, Wahiawa Mountains, Isl. Kauai, August, 1909 (type, Herb. Field Mus.); *Reverend J. M. Lydgate*, same locality (Herb. Bishop Mus.).

EUPHORBIA REMYI **olokelensis** Sk. & Sherff, var. nov.—Folia tenuiter vel sublate petiolata petiolis 4–12 mm. longis; lamina elliptico-oblonga vel subobovato-oblonga, basi saepe inaequilaterali nunc cuneato-angustata nunc subcordata, apice obtusa, membranacea, vix vel non revoluta, 6–10 cm. longa et 2–4.7 cm. lata. (*Cf.* C. SKOTTSBERG, Meddel. Göteborgs Bot. Trädgård 10:121. 1936.)

Specimens examined: *Albert S. Hitchcock* 15220, alt. 1400 ft., Olokele Gulch, Isl. Kauai, Oct. 18, 1916 (Herb. U.S. Nat.); *Joseph F. Rock* 2078, Halemanu, Isl. Kauai, Feb. 14–26, 1909 (Herb. Bishop Mus.; Herb. Gray); *idem* 12936, Kaholuamanu, Isl. Kauai, October, 1916 (Herb. Bishop Mus.); *idem* 17108, same locality and date (Herb. Bishop Mus.); *Dr. Carl Skottsberg* 1050, innermost part of Olokele Valley, Isl. Kauai, Oct. 31, 1922 (type, Herb. Bishop Mus.).

Rock 17108, which, although coming from Kaholuamanu, is so close to the Olokele specimens that it is not varietally separable, has a mature capsule measuring 4 mm. long, the cocci obtuse on the back. The specimens from the type locality are without mature capsules.

The *Skottsberg* specimen had been labeled as new by its collector, Dr. CARL SKOTTSBERG, Director of the Arboretum at Gothenburg, Sweden, and he very courteously permits the publication here of my description of it (this description being extended somewhat to include the other specimens cited).

EUPHORBIA REMYI **wilkesii** var. primum nom.; *Euphorbia remyi* var. *β*. Boiss. *in* DC. Prodr. 15^{11}:1262. 1862.—Folia anguste oblonga, basi inaequilateraliter angustata, apice subacuta acutave raro vix subacuminata membranaceissima, non revoluta, petiolo tenui circ. 7–11 mm. longa, lamina 6–9 cm. longa et 1.5–2.6 cm. lata. Involucrum 2 mm. altum. Capsula tantum circ. 2.2 mm. alta, coccis acute carinatis haud sulcatis, seminis tetraquetri lateribus rugulosis; stipite valde reflexo.

Specimens examined: *United States Exploring Expedition*, Isl. Kauai, 1840 (type, Herb. U.S. Nat.).

ASA GRAY supplied a description of *Euphorbia remyi* to BOISSIER, as is shown in BOISSIER's citation "A. Gray in litt." and the additional note, "Descr. ex cl. A. Gray in litt." BOISSIER published the description in 1862 (*loc. cit.*). As already stated (see p. 584), BOISSIER listed two varieties, a and *β*, basing a on *Remy* 598, "absque fructu," from Oahu, and *β* on the material collected by the *United States Exploring Expedition under Captain Wilkes*, on Kauai. GRAY's description of the species *E. remyi* included the fruit. But since *Remy* 598 was "absque fructu" (and this is confirmed by an examination of GRAY's ample type specimen now before me), the fruit clearly must have been described from that in the packet of fragmentary but fruiting U.S. Explor. Exped. material which GRAY had before him and from which (as stated on the label) he sent "a little to Boissier."

Shortly after BOISSIER's work was published, HORACE MANN botanized in the Hawaiian Islands (May 4, 1864–May 18, 1865). He collected a specimen with larger, more acuminate leaves and capsules 4 (not about 2.2) mm. long, the cocci obtuse (not acutely carinate) upon their backs. ASA GRAY saw this material and his fragment of it, labeled *"Euphorbia Remyi* n. sp." in his own handwriting, is still preserved (in Herb. Gray). He evidently at once revised his supposedly unpublished description of *E. remyi* in an attempt to allow for the larger capsules and MANN, in 1867 (Proc. Amer. Acad.

7:202; Enum. Haw. Pl. no. 438), published the description as revised. Thus we read: "Capsules small, acutely triangular-3-lobed, and glabrous (immature?), or much larger, 2 lines long, the cocci obtuse on the back." The two types of fruit and even of leaves are varietally incongruous and I limit the var. *wilkesii* to plants typified by the original *Wilkes* (small-fruited) material.

EUPHORBIA REMYI hanaleiensis var. nov.—Folia membranacea, petiolo tenui ∓8 mm. longo; lamina late oblanceolato-oblonga, apice subacuminata, ∓12.5 cm. longa. Inflorescentia gracillima, ∓1.7 cm. longa, ramosa, ∓4-cephala, pedicellis tenuibus glabris 4–7 mm. longis; involucro ∓3 mm. lato, superne tomentello; coccis latera versus dense tomentosis, circ. 4 mm. longis; seminibus tetragonis, scrobiculatis, circ. 3 mm. longis.

Specimens examined: *Horace Mann* and *William T. Brigham*, Hanalei, Isl. Kauai, 1864–1865 (type, Herb. Cornell Univ.: cotype fragment, Herb. Gray).

Euphorbia skottsbergii sp. nov.—Frutex manifeste prostratus, ramosus, ramis tenuibus (ultimis tenuissimis) nodosis divaricatis novissimis tomentellis. Folia opposita, petiolo tenui tomentello 1–3 mm. longo; lamina diverse obovata oblonga oblongo-elliptica vel obovato-subrhomboidea, apice obtusa rotundatave, basi saepe inaequilaterali cuneato-rotundata, margine vix subrevoluto plana vel subrepanda (apicem versus rarissime obsoleto-denticulata), membranacea, supra glabrata, infra plus minusve hispidula, plerumque 1–2 cm. longa et 7–13 mm. lata; stipulis interpetiolaribus vix 0.5 mm. altis. Capitula plerumque secundum ramulos minutissimos axillares disposita. Involucrum (non vere pedicellatum) minutum campanulatum, glabratum vel moderate hispidulum, tantum circ. 1–1.1 mm. altum; lobis minutis, erecte oblongis, fimbriatis; glandulis 4 rarius 5, transverse oblongis, exappendiculatis, remotis vel fere contiguis; florum masculorum pedicellis exsertis. Capsula minuta glabrata, tantum circ. 1.6 mm. alta, cernua, breviter hispidula; coccis parce carinatis, non sulcatis; stylis distinctis, fere usque ad medium bifurcatis. Semina ovata, tetragona, basi truncata, apice obtusa, cinerea, papillato-scrobiculata, circ. 1.1 mm. longa.[1]

[1] *Cf.* C. SKOTTSBERG, Meddel. Göteborgs Bot. Trädgård 10:122. 1936.

Specimens examined: *Otto Degener* 8050, arid fossil reef, between Barbers Point and Pearl Harbor, Isl. Oahu, May 8, 1932 (Herb. Degener; Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *Charles N. Forbes* 2330O, coral plain below Ewa and Sisal, Isl. Oahu, Mar. 14, 1916 (Herb. Field Mus., 4 sheets); *Dr. Carl Skottsberg* 122, Ewa coral plain, Isl. Oahu, Aug. 11, 1922 (type, Herb. Bishop Mus.); *O. H. Swezey*, Isl. Oahu (Herb. Bishop Mus.).

The name is given in honor of DR. CARL SKOTTSBERG (Director of the Arboretum of Gothenburg, Sweden), who kindly placed his entire collection of Hawaiian *Euphorbiae* at my disposal for study.

EUPHORBIA SKOTTSBERGII **kalaeloana** var. nov.—Erecta, ramosior, ramulis ultimis saepius capilliformibus. Folia minora, saepius 5–9 (raro –14) mm. longa.

Specimens examined: *Charles N. Forbes* (with *C. M. Cook*) 1760O, near Sisal, Isl. Oahu, Feb. 12, 1912 (Herb. Field Mus.); *Joseph F. Rock* 17034, coral plain under Algaroba, back of Barbers Point, Isl. Oahu, November, 1919 (1st type sheet, Herb. Gray; 2nd type sheet, Herb. Bishop Mus.).

EUPHORBIA SKOTTSBERGII **audens** var. nov.—Folia patentia vel patenti-reflexa, utroque margine plerumque 3–10-denticulata dentulis acribus induratis subpatenti-antrorsis. Semina vix longiora (circ. 1.3 mm. longa).

Specimens examined: *Charles N. Forbes* 620Mo, beach near Ka Lae Ka Ilio Ilio, Isl. Molokai, Mar. 25, 1915 (type, Herb. Bishop Mus.: cotype, Herb. Missouri Bot. Gard.).

EUPHORBIA SKOTTSBERGII **vaccinioides** var. nov.—Folia plerumque elliptica vel anguste obovato-elliptica.

Specimens examined: *Joseph F. Rock* 14072, west end flats, Isl. Molokai, April, 1918 (type, Herb. Bishop Mus.).

Euphorbia festiva sp. nov.—Frutex, unico ramo glabro sulcato sub 1.7 dm. longo viso, nodoso internodiis 1–1.4 cm. longis et 1–2 mm. crassis; ramulis suberectis tenerrimis (0.5–1 mm. crassis) plerumque 0.5–1 cm. longis, angulatis, sulculatis, superne sparsim hispidulis, nodosis nodis duplo crassioribus. Folia disticha, petiolo tenui glabrato tantum circ. 1 mm. longo; lamina oblonga vel rarius ovato-oblonga, apice saepe minutissimo-emarginato obtusa vel subro-

tundata, basi inaequilaterali eleganter lateque subcordata vel etiam moderate cordata, utroque margine angustissime indurata et non vere revoluta sed plerumque obsoletissime 1–8-dentulata, membranacea, utrinque glabra, infra pallidiore, 7–16 mm. longa et 5–11 mm. lata; stipulis interpetiolaribus triangularibus, pubescentibus, sub 1 mm. altis. Capitula terminalia (saltem pro 8 ramulis; nullis lateralibus repertis), solitaria. Involucrum subsessile, campanulatum, extus glabratum vel superne hispidulum, circ. 1.8–2.1 mm. altum; lobis hirtis; glandulis plerumque 5, transverse oblongis, non vel obsolete appendiculatis, plus minusve contiguis; floribus masculis exsertis. Capsula matura non visa; stylis basi connatis, valde bifurcatis ramis incrassatis.

Specimens examined: *Thomas Nuttall*, Isl. Oahu, 1835 (type, Herb. Kew). NUTTALL had designated the type as a new species (*Euphorbia pauciflora* Nutt. *ex* Seem. Fl. Vit. 1:216. 1865–68; *non* Duf. *nec* Hill), but later EDMOND BOISSIER determined it as *Euphorbia multiformis* H. & A. The oblong, basally wide-cordate or wide-subcordate leaves, with their obsolete yet definitely visible teeth, easily distinguish the *Nuttall* plant, however, from that species. Among the many specimens of Hawaiian *Euphorbiae* studied by me from the principal European and American herbaria, no others have been found to belong here, and it may well be that during the century since NUTTALL collected on Oahu the species has become extinct.

EUPHORBIA MULTIFORMIS var. **sparsiflora** (Heller) comb. nov.; *E. sparsiflora* Heller, Minnesota Bot. Studs. 1:846, pl. 51. 1897; *E. palustris* Heller, *loc. cit.* 847.

EUPHORBIA MULTIFORMIS **kaalana** var. nov.—Ramuli tenues sed perspicue nodosi, tomentelli, internodiis numerosis saepe tantum 3–6 mm. longis. Foliorum petioli tomentelli circ. 1–1.5 mm. longi; lamina plus minusve obovata, membranacea, basi inaequilaterali, apice rotundata saepe etiam retusa, subtus argenteo-tomentella, sub 2 cm. longa, venis obsoletissimis. Capitula numerosiora, saepe ad ramuli minimi axillaris apicem 2–5-congregata, perspicue tomentosa, circ. 3 mm. lata, glandulis brunneis plus minusve glabratis, antheris numerosissimis. Capsulae pilosulae, circ. 2.5 mm. altae et circ. 3 mm. latae. Semina subtetragona, scrobiculata, 1.5 mm. longa.

Specimens examined: *United States Exploring Expedition under Captain Wilkes*, "Kaala Mts., Wai-Nai," Isl. Oahu, 1840 (type, Herb. U.S. Nat.: cotype fragments, Herb. Bishop Mus.; Herb. Gray; Herb. Missouri Bot. Gard.).

A transitional form which might almost as well be considered a variety of *E. celastroides* Boiss. It was confused by ASA GRAY with *E. multiformis* var. *tomentella* Boiss., a plant with leaf blades mostly 2-3 cm. long, their veins conspicuous underneath, internodes of branchlets more elongate, capitula singly disposed, stamens fewer, etc.

EUPHORBIA MULTIFORMIS **haleakalana** var. nov.—Habitu varietati *microphyllae* Boiss. simillima. Rami foliorum petiolique hispidi. Involucrum extus nunc glabratum nunc (praecipue summam versus) patenti-hispidum; glandulis saepius patentibus; pedicella dense divaricateque albo-hispida. Capsula circ. 1.5 mm. alta, stipite patenti-hispido.

Specimens examined: *Charles N. Forbes 2010M*, Auwahi, south slope of Haleakala, Isl. Maui, Mar. 18, 1920 (type, Herb. Field Mus.).

EUPHORBIA MULTIFORMIS **kapuleiensis** Degener & Sherff, var. nov.—Habitu varietati *microphyllae* similis. Frutex circ. 1.8 m. altus, gracillimus, ramis elongatis subpendulisque, ramulis ultimis subcapilliformibus vel etiam capilliformibus; internodiis plerumque elongatioribus; foliis plerumque lineari-oblongis vel anguste ellipticis, petiolo 2-3.5 mm. longo, lamina sub 3 cm. longa.

Specimens examined: *Otto Degener 8053*, 6 ft. tall with long, almost drooping branches, rare, up ridge called Kapulei to east of white mountain Kaholoapele and back in east gully, Isl. Molokai, June 25, 1928 (type, Herb. Delessert: cotypes, Herb. Degener; Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *idem 8056*, on arid cliffs, second eastern gulch, Wawaia, Isl. Molokai, June 27, 1928 (Herb. Degener, 3 sheets; Herb. Field Mus.); *idem 8061*, one of the dry valleys between Kamalo and Kaunakakai, Isl. Molokai, Jul. 29, 1928 (Herb. Degener, 2 sheets; Herb. Delessert; Herb. Field Mus., 2 sheets).

EUPHORBIA MULTIFORMIS **manoana** var. nov.; *E. multiformis* Gaud. (*saltem pro magna parte*), Bot. Freycin. Voy. 100. 1830 (*nom.*

subnudum); Boiss. *in* DC. Prodr. 15^{II}: 11. 1862 (*ex plantis Eschscholtzii, Maximoviczii, Macraeique, etiam descriptione et syn. Gaud.; excl. syn. Hook. & Arn.*).—Rami juniores glabri (pro typo) vel patenti-hispiduli. Folia saepius elliptico-oblonga vel cuneate oblanceolata, apice plerumque rotundata vel truncato-rotundata et saepe retusa, interdum facie inferiore hispidula et saepe petiolo tomentulosa. Involucrum extus basim versus papillato-hirtum sed summam versus dense tomentosum.

Specimens examined: *Otto Degener H*-213, on Tantalus side of Manoa Valley, Isl. Oahu, Feb. 12, 1923 (Herb. Degener, 2 sheets; Herb. Field Mus.; Herb. N.Y. Bot. Gard., 2 sheets); *Frederick Eschscholtz*, Isl. Oahu, 1816–1817 (Herb. Delessert; Herb. Gray; Herb. Kew); *Charles N. Forbes*, ridge west of Kalihi Valley, Isl. Oahu, Aug. 18, 1908 (Herb. Field, 2 sheets; Herb. Missouri Bot. Gard.); *idem* 358*H*, growing 12 ft. tall, with drooping branches, Kanehaha, Kona, Isl. Hawaii, Jul. 25, 1911 (Herb. Bishop Mus.); *idem* 1660*O*, Pacific Heights ridges, Isl. Oahu, March, 1911 (Herb. Field Mus., 2 sheets); *Charles Gaudichaud*, Hawaiian Isls., 1819 (Herb. Berlin); *Dr. William Hillebrand*, Honolulu, Isl. Oahu (Herb. Mus. Vienna); *Lay* and *Collie* (*Capt. Beechey's Voyage*), Isl. Oahu, 1826–1827 (Herb. Mus. Vienna); *James Macrae*, Isl. Oahu, May 20, 1825 (Herb. Berlin; Herb. N.Y. Bot. Gard.; Herb. Mus. Vienna); *Maximovicz* 145, Honolulu, Isl. Oahu (type, Herb. Kew: cotype, Herb. Berlin); *George C. Munro* 69, Maluea, Isl. Lanai, Dec. 27, 1913 (Herb. Bishop Mus.); *idem* 80, *eodem loco et tempore* (Herb. Bishop Mus., 2 sheets); *Joseph F. Rock*, right-hand branch of Wailupe Valley, Isl. Oahu, Apr. 14, 1918 (Herb. Bishop Mus.); *idem* 8126, Koele, Isl. Lanai, Aug. 3, 1910 (Herb. Field Mus.); *Carl Skottsberg* 1073, Nuuanu-Pauoa ridge, Isl. Oahu, Nov. 5, 1922 (Herb. Bishop Mus.); *Adelbert Von Chamisso* 182, Isl. Oahu, 1816–1817 (Herb. Berlin; Herb. Leningrad).

BOISSIER, to whom this variety represented the species proper, described the plants as glabrous. In two of the collections cited by him (*Eschscholtz's* and *Macrae's*), however, the younger branchlets and leaf petioles and lower surfaces are more or less hispidulous; so also for at least some of Gaudichaud's original material, of which I have seen KUNTH's ample fragment (Herb. Berlin). The difference

in pubescence seems hardly constant enough to justify attempts at
further than varietal delimitation.

EUPHORBIA CELASTROIDES var. **stokesii** (Forbes) comb. nov.; *E.
stokesii* Forbes, Bishop Mus. Occas. Papers 5:108, pl. 1. 1913.

EUPHORBIA CELASTROIDES **moomomiana** var. nov.—Multum
ramosa; ramis rubidis, crassis, conferte foliatis, internodiis ple-
rumque 3–6 mm. crassis et duplo triplove longis. Folia perspicue
disticha imbricataque, opposita, saepius reflexa; lamina subrigida,
obovata, apice rotundata vel truncata interdum emarginata (raro
etiam utroque latere apicem versus minute 1-sinuata), usque ad
basim minute auriculato-cordatam sensim angustata, margine in-
tegra et subrevoluta, caeruleo-viride, infra pallidiore, 3 -4.8 cm.
longa et 1.8–2.8 cm. lata; corpore stipulari marginaliter fimbriato-
hispido, 1.5–3.2 mm. alto. Capitula solitaria axillariaque vel 2- 5
secundum axem incrassatum brevemque ex axilla ortum; pedicella
glabra, ∓1 cm. longa. Capsula circ. 2 mm. alta et fere 3 mm.
crassa.

Specimens examined: *Edward L. Caum* 10, Kaula, Hawaiian
Isls., Aug. 17, 1932 (Herb. Bishop Mus.); *Otto Degener* 8069, forming
1 × 2 yard mass, in pure sand on leeward side of large dune, extreme-
ly dry region, Moomomi, Isl. Molokai, Apr. 25, 1928 (Herb. De-
gener, 2 sheets; Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *George C.
Munro* 494, Moomomi sand hills, Isl. Molokai, Jul. 26, 1922 (Herb.
Bishop Mus.); *Joseph F. Rock* 14014, Moomomi, Isl. Molokai, April,
1918 (type, Herb. Bishop Mus., 3 sheets).

The *Munro* plant appears to have been regarded as new by ROCK,
but at the Bishop Museum both the *Munro* and the *Rock* plants
had been referred to the deceptively similar *Euphorbia stokesii* (my
var. *stokesii*). The latter plant differs strongly, however, in having:
rameal internodes much shorter and thicker; the much more numer-
ous leaves so close that in the herbarium specimens they present a
highly imbricated effect, their blade only very rarely with an inden-
tation on each side of and a few millimeters from the midnerve's
distal end; the tendency to bear thickened, abbreviated, axillary
branches which are mere axes to hold the capitula; the shorter cap-
sule, etc.

EUPHORBIA CELASTROIDES **kaenana** var. nov.—Frutex, ramis crassis et valde nodosis, cortice saepe albo-griseo et demum valde rugoso. Folia opposita, disticha, secundum ramulorum ultimorum brevissimorum 2–4 mm. crassorum partem tertiam (vel dimidiam) superiorem moderate conferta, inferiora saepe reflexa; petiolo gracili, glabro, 2–3 mm. longo; lamina late vel anguste oblongo-oblanceolata, apice obtusa vel truncato-subemarginata, inferne usque ad basim inaequilateralem truncato-subcordatam 3–5 mm. latam cuneate vel subcuneate angustata, pallide viridi, membranacea, plerumque 3–4.5 cm. longa et 1–1.6 cm. lata. Cymae multum contractae, axe ramisque crassiusculis nodosisque (bracteis stipularibus numerosis perspicuissimis); capitulis saepe 5–7, sessilibus vel subsessilibus; involucro extus glabrato vel minute setuloso nisi lobis hirsutis. Capsula subsessilis, glabrata, demum subnigra, circ. 2.5 mm. alta; seminibus extus subgriseis (subextus rubidis), biconvexo-tetragonis, scrobiculatis, apice baseque inaequilateralibus, tetragonis, 1.2–1.4 mm. longis.

Specimens examined: *Otto Degener, W. Hirai,* and *Kwan Kee Park* 8038, among rocks in arid region, near Kaena Point, Isl. Oahu, Mar. 21, 1931 (Herb. Degener; Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *Charles N. Forbes* 16540, between Makua Valley and Kaena Point, Isl. Oahu, Feb. 25, 1911 (Herb. Field Mus.); *idem (cum Dean Lake)* 22800, talus slopes, Kaena Point, Isl. Oahu, Dec. 16, 1915 (Herb. Bishop Mus.); *Vaughan MacCaughey,* Kaena uplands, Isl. Oahu, Mar. 28, 1915 (type, Herb. Bishop Mus.).

EUPHORBIA CELASTROIDES **waikoluensis** var. nov.—Frutex, ramis subrubris moderate tenuibus. Folia moderate numerosa sed raro imbricata, pallida vel glaucescentia; petiolo subatro, minute puberulo, tantum circ. 1–2 mm. longo; lamina subrhombico-ovali, apice obtusa vel rotundata et interdum subemarginata, basi lata (4–8 mm.) et truncato-subcordata, plerumque 2.5–3.8 cm. longa et 1.5–2.5 cm. lata. Capitula (pauca visa) solitaria vel subsolitaria, axillaria vel terminalia; involucro praecipue summam versus pubescenti; pedicella brevi (1–3 mm.). Capsula (unica immatura visa) nigra.

Specimens examined: *Joseph F. Rock* 6191, on beach of Waikolu, Isl. Molokai (type, Herb. Field Mus.).

EUPHORBIA CELASTROIDES **haupuana** var. nov.—Sine dubio fruticosa; ramis ligneis, gracilibus, griseo-nigris, ultimis numerose

perspicueque nodosis (internodiis plerumque 3–7 mm. longis et 1–2.5 mm. crassis). Folia ut apud speciem ipsam (sed lamina minora et plerumque sub 3 cm. longa et 2 cm. lata apice plerumque obtusa rarissime retusa), ad ramorum apices subconferta. Capitula plerumque tantum 1 vel 2 ad unicum nodum; pedicella tenui, glabra, 4–7 mm. longa. Capsula (unica valde immatura visa) nigra.

Specimens examined: *Charles N. Forbes* 20K *pro parte,* near Lihue, Isl. Kauai, Jul. 9, 1909 (Herb. Field Mus.); *idem* 600K, Nonou Mountains, Isl. Kauai, Oct. 16–17, 1916 (Herb. Field Mus.); *Joseph F. Rock* 2444 "Haupu Lihue," Isl. Kauai, Mar. 18, 1909 (type, Herb. Bishop Mus.: cotype, Gray).

EUPHORBIA CELASTROIDES **kohalana** D. & S. var. nov.—Frutex, laxe ramosus; ramis subrubris, tenuibus, internodiis longis (saepe 2–4 cm.), nodis non perspicuis; ramis ultimis tenuissimis (∓1 mm. crassis), moderate foliatis et saepe ad apicem etiam ad 1 vel 2 nodos 1 vel 2 capitula brevissimi-pedicellata (pedicella 1–3 mm. longa) gerentibus. Folia angustius petiolata; lamina ovali vel obovata (raro paulum subrhomboidea), caeruleo-viridi vel glaucescenti, apice rotundata vel emarginata, basi plus minusve inaequilaterali anguste vel late rotundata vel truncato-subcordata, 2.5–4 cm. longa et 1.5–2.5 cm. lata.

Specimens examined: *Otto Degener* 8037, Kohala, Isl. Hawaii, Mar. 22, 1930 (type, Herb. Delessert: cotypes, Herb. Field Mus., 2 sheets; Herb. N.Y. Bot. Gard.); *Joseph F. Rock* 14032, without data (Herb. Bishop Mus.; a specimen without flowers or fruits and with the leaves mostly less blunt or rounded at apex).

EUPHORBIA CELASTROIDES **kealiana** var. nov.—Probabiliter fruticosa et mattae plus minusve similis; ramis subnigris, nodis numerosis perspicuisque, internodiis nunc 3–7 mm. nunc ∓1 cm. longis et ∓3 mm. (vel ramorum ultimorum 1–1.5 mm.) crassis. Folia ut apud speciem ipsam sed demum magis membranacea, lamina usque ad 5 cm. longa et 3.3 cm. lata, colore probabiliter diversa. Capitula non visa.

Specimens examined: *Abbé Urbain Faurie* 477, on rocks of the shore at Kealia, Isl. Kauai, January, 1910 (type, Herb. Brit. Mus.); *Charles N. Forbes* 20K *pro parte,* near Lihue, Isl. Kauai, Jul. 9, 1909 (Herb. Field Mus.).

EUPHORBIA CELASTROIDES **halawana** var. nov.—Frutex ramosissimus foliosissimus; ramis ultimis subultimisque subrubris longitudinaliter valde rugosis (saltem demum), internodiis plerumque 1–2 mm. crassis. Folia minus decidua, demum subbrunnea, multo minora; petiolo tenui, 1–2 (raro –3) mm. longo; lamina diverse ovali obovata subrhomboideo-obovata vel suborbiculari, apice rotundata vel truncato-emarginata, basi inaequilaterali et plerumque sublata rotundata vel truncato-subcordata, pro paucis foliis caulis usque ad 3.5 cm. longa et 2 cm. lata sed pro multitudine foliorum ramorum plerumque 1–2.5 cm. longa et 0.8–2 cm. lata (vel pro minutis saepe numerosis foliis capitula subtendentibus ∓4 mm. longa et ∓4 mm. lata). Capitula subsessilia, plerumque solitaria, saepe ad et prope summam ramuli lateralis pumili folioso-bracteolati gesta; involucro saltem supra extus tomentoso. Capsula glabra vel aegre pubescens, viridi-brunnea, sessilis vel subsessilis, ∓3 mm. alta; seminibus subgriseis, scrobiculatis, 2 mm. longis.

Specimens examined: *Otto Degener* 8054, dry coastal cliffs, west side of Halawa Valley, Isl. Molokai, June 20, 1928 (Herb. Degener, 3 sheets; Herb. Field Mus., 2 sheets); *idem et Ross S. Bean* 8041, Isl. Oahu, Apr. 12, 1931 (Herb. Degener); *Degener* and *Noel Krauss* 8036, sunny ridge, west slope of Kahana Valley, Isl. Oahu, Feb. 26, 1929 (Herb. Degener; Herb. Field Mus.; Herb. N.Y. Bot. Gard.; type of forma *kahanana, ramulis siccis minus rugosis costatisve, foliis numerosioribus, inflorescentiis saepius foliis minimis subtentis*); *Abbé Urbain Faurie* 472, on shore at Halawa, Isl. Molokai, June, 1909 (Herb. British Mus.); *Joseph F. Rock* 14041, near Halawa, Isl. Molokai, April, 1918 (type and cotype, Herb. Bishop Mus.).

EUPHORBIA CELASTROIDES **saxicola** Degener & Sherff, var. nov.—Habitu var. *halawanae* moderate similis. Folia plerumque paulo minora et saepius obovata, apice emarginata. Involucrum plerumque breviter (1–4 mm.) sed manifeste pedicellatum. Capsula (sessilis) demum brunneo-atra vel atra.

Specimens examined: *Otto Degener* 8088, along rocky shore, Kohala, Isl. Hawaii, Aug. 9, 1926 (type, Herb. Delessert; cotypes, Herb. Degener, 2 sheets; Herb. Field Mus.).

EUPHORBIA CELASTROIDES **humbertii** var. nov.—Probabiliter fruticosa; ramis adscendentibus, tenuibus, ultimis plerumque ad

internodia (saepe 3–9 mm. longa) 1–1.6 mm. crassis, nodis perspicuis. Folia moderate conferta secundum tertiam vel dimidiam superiorem ramulorum, petiolo tenui 2–4 mm. longo; lamina cuneate et saepe subfalcate oblanceolata, apice obtusa vel truncata vel rotundata sed raro retusa, basi inaequilaterali-truncata et tantum circ. 1.5–3 mm. lata, caerulea vel glaucescenti, submembranacea, plerumque 2–4 cm. longa et o.7–1.5 cm. lata. Capitula multa sed plerumque tantum 1 vel 2 ad unum nodum, plerumque elongato-pedicellata; involucro extus glabrato; pedicella tenui, rigida, suberecta et demum ∓13 mm. longa. Capsula glabra, demum brunneo-atra, circ. 2.5 mm. alta.

Specimens examined: *Jules Remy* 595, Kauai, 1851–1855 (type, Herb. Gray: cotypes, Herb. Paris, 2 sheets).

Named for PROFESSOR H. HUMBERT of Paris, without whose invaluable assistance my treatments of this and several other members of the genus *Euphorbia* would have been greatly handicapped, if not indeed precluded.

EUPHORBIA CELASTROIDES **nematopoda** var. nov.—Habitu var. *humbertii* similis; foliis minus confertis, capitulis paucioribus et in pedicellis parte tertia vel dimidia longioribus etiam gracilioribus magis flexilibus non rigidis suberectisque.

Specimens examined: *Charles N. Forbes* 726K, at left-hand side of Kipu Kai Gap, Haupu Range, Isl. Kauai, Nov. 1, 1916 (type, Herb. Bishop Mus.).

EUPHORBIA CELASTROIDES var. **lorifolia** (A. Gray) comb. nov.; *E. multiformis* var. *lorifolia* A. Gray *ex* H. Mann, Proc. Amer. Acad. 7:202 (Enum. Haw. Pl., no. 439, var. δ). 1867; *E. lorifolia* Hillebr. Fl. Haw. Isls. 395. 1888 (*ex syn. A. Grayi*); *E. rivularis* Heller, Minn. Bot. Studs. 1:846, pl. 51. 1897.

EUPHORBIA CELASTROIDES **hanapepensis** var. nov.; *E. celastroides* Heller, Minn. Bot. Studs. 1:844. 1897 (*non* Boiss.).—Arbor parva, laxe ramosa, caule brevi; ramis minoribus vel junioribus rigidis et internodiis brevibus (plerumque 5–12 mm.), subbrunneo-atris vel saltem demum subgriseo-atris. Folia ramulorum apices versus subconferta, petiolo tenui et 1–4 mm. longo; lamina lineari-oblanceolata vel late oblanceolata, apice rotundata vel subobtusa, basi angusta (plerumque sub 3 mm. lata), magis membranacea et supra subviridiore, infra moderate flavido-argentea, 2–5 cm. longa et o.7–1.7 cm.

lata. Capitula multo numerosiora quam apud speciem ipsam, solitaria ad nodos vel saepius in cymis 2–5-cephalis et in ramulorum lateralium foliosorum brevium axillis disposita; involucro extus glabro; pedicella tenui, glabra, 3–8 mm. longo. Capsula glabra, demum atro-brunnea, seminibus subgriseis, scrobiculatis.

Specimens examined: *Abbé Urbain Faurie* 476, on rocks, Hanamaulu, Isl. Kauai, December, 1909 (Herb. Bishop Mus.; Herb. Delessert); *A. A. Heller* 2429, along the Hanapepe River, near the Falls, Isl. Kauai, June 24–26, 1895 (Herb. Field Mus., 2 sheets; Herb. Gray; Herb. N.Y. Bot. Gard.); *Heller* (*similiter*) 2429, on the Hanapepe and Wahiawa Watershed, Isl. Kauai, June 24, 1895 (Herb. Kew); *Heller* (*similiter*) 2429, along the Hanapepe River, near the Falls, Jul. 2–8, 1895 (type, Herb. Missouri Bot. Gard.: cotype, Herb. U.S. Nat.).

EUPHORBIA CELASTROIDES **niuensis** var. nov.—Varietati *lorifoliae* similis. Foliorum petioli tenues, glabrati, 2–3 mm. longi; lamina oblonga, basi inaequilaterali-truncata et 3–5 mm. lata, apice obtusa rotundatave, glaucescenti, subtenui, ∓2.5 cm. longa et ∓1.2 cm. lata. Capitula ∓11-adgregata in cymulis 1.5 cm. longis, cymulorum ramulis patentibus vel recurvatis, pedicellis tenuibus sed brevibus (1–4 mm. longis). Capsulae siccae atro-brunneae, glabrae, sessiles vel subsessiles, circ. 2 mm. altae. Semina extus subgrisea subextus rubida, tetragona, tantum circ. 1–1.1 mm. longa, tantum moderate vel etiam indistincte scrobiculata.

Specimens examined: *Dr. William Hillebrand*, Niu, Isl. Oahu (type, Herb. Berlin).

Perhaps now extinct. The type is a mere fragment but very distinctive. It is the specimen referred to by HILLEBRAND (Fl. Haw. Isls. 395. 1888) for the Oahu material of *Euphorbia celastroides*. I have found no other material to match it.

EUPHORBIA CELASTROIDES **amplectens** var. nov.; *E. lorifolia* Hillebr. Fl. Haw. Isls. 395. 1888 (*pro parte parva et exclud. syn.* A. Gray *et* DC.; *cf. var. mauiensem*).—Nunc frutex prostratus erectusve nunc arborescens, ramosissima; ramis ultimis penultimisque griseoatris, puberulis, non valde rugosis, nodis numerosis perspicuis, internodiis plerumque 1–2 mm. crassis. Folia polymorpha sed plerumque petiolo angusta pubescentia 1–3 mm. longa; lamina plerumque ellip-

tico-ovata vel anguste oblanceolata, apice truncata (etiam emargi-
nata) vel rotundata vel obtusa, basi rotundata vel angustata, viridi
vel demum plus minusve brunnescenti, infra interdum (saltem peti-
olum versus) puberula, plerumque, 1.5–3 cm. sed interdum usque ad
6 cm. longa. Capitula plerumque solitaria, subsessilia; involucro ex-
tus basim versus glabro vel glabrato summam versus saepe pubescen-
ti vel perspicue tomentoso. Capsula glabra vel moderate puberula,
sessilis vel subsessilis, demum (sicca) brunneo-viridis vel atro-brun-
nea, circ. 3 mm. alta. Semina nunc manifeste tetragona nunc ob-
compressa et (basi truncata tetragonaque excepta) 2-marginata,
apice obtusa, extus grisea subextus rubida, scrobiculata, 1.7–2 mm.
longa.

   Specimens examined: *Anon.* 375, shrub about 4–6 ft. tall, Pololu
Gulch, Kohala, Isl. Hawaii (Herb. Field Mus.); *Otto Degener* 8043,
windswept ridge at beginning of forest, east ridge of Niu Valley, Isl.
Oahu, Apr. 20, 1931 (Herb. Degener; Herb. Field Mus.; Herb. N.Y.
Bot. Gard.); *idem* 8055, growing 3.5–5 ft. tall, with *Santalum*, Moko-
moko Gulch, Isl. Molokai, June 7, 1928 (Herb. Degener); *idem* 8057,
shrub 3 ft. tall, with *Lipochaeta degeneri*, in hot boulder-strewn re-
gion not far from ocean, near Kamakaipo, Isl. Molokai, May 16,
1928 (Herb. Degener); *idem* 8058, on moderately dry rocky slope,
growing with *Exocarpus*, Kahuaawi Gulch, Isl. Molokai, May 12,
1928 (Herb. Degener; Herb. Field Mus.; Herb. N.Y. Bot. Gard.);
idem 8060, East Ohia ridge, Isl. Molokai, Jul. 17, 1928 (Herb. De-
gener; Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *idem* 8062, one of
the dry valleys between Kamalo and Kaunakakai, Isl. Molokai, Jul.
29, 1928 (Herb. Degener; Herb. Field Mus.; Herb. N.Y. Bot. Gard.);
idem 8076, dry rocky region, Kaupo Gap, Haleakala, Isl. Maui, Aug.
20, 1927 (Herb. Degener; Herb. N.Y. Bot. Gard.); *idem* 8077, north-
west of Mt. Eke, Isl. Maui, Aug. 31, 1927 (Herb. Degener, 2 sheets;
Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *idem* 8078, arid rocky re-
gion, Ulupalakua, Isl. Maui, June 23, 1927 (Herb. Degener, 2 sheets;
Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *idem* 8087, arid *aa* lava
desert, Hoopuloa, Isl. Hawaii, Aug. 26, 1926 (Herb. Degener, 3
sheets; Herb. Field Mus.); *idem* 8089, in tapestry forest, Kohala
ditch trail, Kohala, Isl. Hawaii, Aug. 10, 1926 (Herb. Degener, 3
sheets; Herb. Field Mus.); *idem et Kwan Kee Park* 8045, in arid

rocky sunny region, second ridge east of Kuliouou Valley, near sum-
mit (ridge on west side of Kamehameha Farm School), Isl. Oahu,
Oct. 25, 1931 (Herb. Degener, 2 sheets; Herb. Field Mus.);
David Douglas 13, Hawaiian Isls., 1834 (Herb. Kew, 2 sheets);
Frederick Eschscholtz, Isl. Oahu, 1816–1817 (Herb. Kew; *forma foliis
E. multiformi valde adpropinquans); Abbé Urbain Faurie* 471, on
rocks, Yao Valley, Isl. Maui, August, 1909 (Herb. Brit. Mus.);
Charles N. Forbes 17*Mo*, Isl. Molokai, June, 1912 (Herb. Bishop
Mus.); *idem* 43*L*, mountains near Koele, Isl. Lanai, June, 1913
(Herb. Field Mus., 2 sheets); *idem* 93*M*, Iao Valley, West Maui,
June, 1910 (Herb. Field Mus.); *idem* 314*L*, Isl. Lanai, September,
1917 (Herb. Field Mus.); *idem* 355*Mo*, Kaluoohu Valley, Isl. Molo-
kai, August, 1912 (type, Herb. Field Mus.: cotypes, Herb. Field
Mus.; Herb. Missouri Bot. Gard.); *idem* 358*H*, Kanehaha, Kona,
Isl. Hawaii, Jul. 25, 1911 (Herb. Bishop Mus.); *idem* 480*M*, Honaka-
hau Drainage Basin, Isl. Maui, Sept. 25–Oct. 17, 1917 (Herb. Field
Mus.); *idem* 533*Mo*, Halawa, Isl. Molokai, September, 1912 (Herb.
Field Mus.); *idem* 1511*O*, Koko Head, Isl. Oahu, June 11, 1909
(Herb. Bishop Mus.); *idem* 1666*O*, Puu-O-Kona, Isl. Oahu, Mar. 14,
1911 (Herb. Field Mus.); *idem* 1775*M*, Isl. Maui, Dec. 9, 1919
(Herb. Bishop Mus.); *idem* 1913*M*, Nuu, south slope of Haleakala,
Isl. Maui, Mar. 9, 1920 (Herb. Field Mus.); *idem* 2386*M*, Olowalu
Valley, Isl. Maui, May 16, 1920 (Herb. Field Mus.); *idem* 2529*O*,
Wailupe, Isl. Oahu, January, 1919 (Herb. Bishop Mus.); *Charles
Gaudichaud*, Hawaiian Isls. (Herb. Missouri Bot. Gard., fragment
ex Herb. Delessertii); Dr. William Hillebrand, Isl. Molokai, June 21
(Herb. Berlin; labeled *Euphorbia lorifolia* by HILLEBRAND); *idem* 48,
Isl. Hawaii (Herb. Kew); *Albert S. Hitchcock* 14484, shrub or small
tree on lava flow, Puuwaawaa, Isl. Hawaii, Aug. 30, 1916 (Herb.
Bishop Mus.; Herb. U.S. Nat.); *James Macrae*, Isl. Maui, May, 1825
(Herb. Missouri Bot. Gard.; fragment *ex herb. Kunthii); idem.* Isl.
Oahu, May 20, 1825 (Herb. N.Y. Bot. Gard.); *Horace Mann* and
William T. Brigham 101 *pro parte*, Isl. Oahu, 1864–1865 (Herb.
Field Mus.); *iidem* 427, ridge east of Nuuanu Valley, Isl. Oahu,
1864–1865 (Herb. Bishop Mus.); *Maximowitsch* 145, Honolulu, Isl.
Oahu (Herb. Berlin; Herb. Kew); *George C. Munro* 45, outer forest,
Kaiholena, Isl. Lanai, August, 1913 (Herb. Bishop Mus.); *Joseph F.*

Rock, Hawaiian Isls. (Herb. Field Mus., 2 sheets); *idem*, Honakahau Valley, Isl. Maui (Herb. Bishop Mus.); *idem* 8068, Hawaiian Isls. (Herb. Field Mus.); *idem* 8126, Koele, Isl. Lanai, Aug. 3, 1910 (Herb. Gray); *idem* 8359, Hawaiian Isls. (Herb. Field Mus.); *idem* 8677, East Maui (Herb. Field Mus.); *idem* 8679, *eodem loco* (Herb. Field Mus.); *idem* 17036, prostrate, near the ocean, Barbers Point, Isl. Oahu, November, 1919 (Herb. Bishop Mus., 2 sheets; Herb. Gray); *Dr. Carl Skottsberg* 1953, along road between Lind's and Pauwaawaa, Isl. Hawaii, Sept. 26, 1926 (Herb. Bishop Mus.); *John F. G. Stokes*, Isl. Molokai, 1909 (Herb. Bishop Mus.); *D. LeRoy Topping* (*Otto Degener* distrib. no.) 8075, Isl. Maui, Aug. 5, 1927 (Herb. Degener; Herb. Field Mus., 2 sheets); *Adelbert Von Chamisso*, Isl. Oahu, 1816–1817 (Herb. Missouri Bot. Gard., fragment *ex Herb. Berol.;* Herb. Petrop., 2 sheets); *Dr. Heinrich Wawra* 1852, Isl. Maui, 1868–1871 (Herb. Mus. Vienna, 2 sheets).

Similar to and apparently passing by many intergradations into the var. *mauiensis*, but with leaves tending to be more often obovate and less often linear, and with seeds commonly larger (1.7–2 mm. not 1.4–1.6 mm. long) and usually tetragonal (only rarely—for example, *Rock* 17036—obcompressed). Plants perhaps not averaging as tall as in var. *mauiensis*, but some of the specimens included here are from plants ∓3.6 m. tall (for example, *Forbes* 358*H*, "slender, 12 ft., drooping branches").—Apparently consists of several *formae* or races.

EUPHORBIA CELASTROIDES mauiensis var. nov.; *E. lorifolia* Hillebr. Fl. Haw. Isls. 395. 1888 (*pro parte magna et exclud. syn.* A. Gray *et* DC.; *cf. var. amplectentem*).—E frutice altitudinum minorum usque ad arborem parvam (3–6 m. altam et cum caule 1.5–2 dm. crasso) altitudinum majorum; ramis rigidis, nodosis, puberulis, internodiis brevibus. Folia breviter (1–2 mm.) petiolata, petiolo puberulo; lamina lineari vel lineari-oblonga vel rhomboideo-lineari, apice obtusa vel truncata et saepe retusa, basi paulum contracta etiam subtruncata inaequilateralique, glabra vel infra praecipue basim versus moderate puberula, 3–5 (rarius –7 vel etiam –9.5) cm. longa, demum (sicca) plus minusve brunneo-viridi, venis lateralibus ventraliter atris distinctisque. Capitula plerumque solitaria, sessilia subsessiliave; involucro extus pubescenti. Capsula puberula, sub-

sessilis, profunde 3-divisa, demum (sicca) brunneo-viridis vel atro-
brunnea, ∓2.7 mm. alta. Semina nunc obcompresso-tetragona (2
marginibus interdum ad costas medianas reductis) nunc manifeste
obcompressa et lateraliter tantum (nisi basi tetragona) 2-marginata,
basi truncata et apice leviter rotundata, brunneo-grisea vel metal-
lico-brunnea, scrobiculata, 1.4–1.6 mm. longa.

Specimens examined: *Anon.*, Isl. Maui (Herb. Bishop Mus.,
fragment *ex Herb. Berol.*); *Otto Degener* 8074, barren arid hill, *mauka*
of McGregor, West Maui, Jul. 10, 1927 (Herb. Degener; Herb. N.Y.
Bot. Gard.); *Charles N. Forbes*, 115*L* mountains near Koele, Isl.
Lanai, June, 1913 (Herb. Bishop Mus.); *idem* 139*L*, *eodem loco et
tempore* (Herb. Field Mus.); *idem* 223*L*, mountains at east end of
Lanai, *eodem tempore* (Herb. Bishop Mus.; Herb. Field Mus.); *idem*
366*L*, Kaiholena, Isl. Lanai, September, 1917 (Herb. Field Mus., 2
sheets); *idem* 1104*M*, Kaupo Gap, Haleakala Crater, Isl. Maui,
Aug. 10, 1919 (Herb. Field Mus.); *idem* 1811*M*, Kanaio, south slope
of Haleakala, Isl. Maui, Mar. 2, 1920 (Herb. Bishop Mus.); *idem*
1812*M*, *eodem loco et tempore* (Herb. Field Mus.); *idem* 2048*M*,
Auwahi, south slope of Haleakala, Isl. Maui, Mar. 20, 1920 (Herb.
Field Mus.); *idem* 2091*M*, Auhi, south slope of Haleakala, Isl.
Maui, Mar. 24, 1920 (Herb. Field Mus.); *idem et C. Montague Cook
Jr.* 1*M*, Maunahooma, West Maui, May, 1910 (Herb. Bishop Mus.);
Dr. William Hillebrand 45, small tree 15–20 ft. tall, Kula, East
Maui, July, 1858 (Herb. Kew, 2 sheets); *idem et Rev. J. M. Lydgate*,
Hawaiian Isls. (Herb. Bishop Mus.); *Albert S. Hitchcock* 14809, alt.
3000–5000 ft., Puu Kukui, West Maui, Sept. 24–26, 1916 (Herb.
Bishop Mus.; Herb. U.S. Nat.); *Horace Mann* and *William T.
Brigham* 389, sandy isthmus of Maui, 1864–1865 (type, Herb. U.S.
Nat.: cotypes, Herb. Bishop Mus.; Herb. Field Mus.; Herb. Gray;
Herb. Missouri Bot. Gard.); *George C. Munro* 66, Kaiholena, Isl.
Lanai, August, 1913 (Herb. Bishop Mus.); *idem* 131, outer forest,
ridge behind Kaiholena, Isl. Lanai, Sept. 28, 1913 (Herb. Bishop
Mus.; Herb. Field Mus.); *Joseph F. Rock*, 8073, Mahana, Isl. Lanai,
July, 1910 (Herb. Field Mus., 2 sheets; Herb. Gray; Herb. Mus.
Vienna); *idem* 8560, gulches above Makawao, Isl. Maui, September,
1910 (Herb. Field Mus.; Herb. Gray; Herb. N.Y. Bot. Gard.); *Dr.
Heinrich Wawra* 2343, Hawaiian Isls., 1868–1871 (Herb. Mus. Vien-

na); *idem* 2527, Isl. Maui, *eodem tempore* (Herb. Mus. Vienna); *Garrit P. Wilder*, Isl. Maui, 1913 (Herb. Bishop Mus.).

As stated under the foregoing variety (var. *amplectens*), that variety apparently passes into this. Because of the peculiarly obcompressed seeds, I had originally held this to be a valid species. The presence of forms of var. *amplectens*, however, in which forms most of the seeds are distinctly obcompressed (although still of greater length), seems to cast doubt upon the value of the obcompressed-seed character. Anyway, the local and visiting botanists who have collected the two forms have very commonly confused them under the single though erroneous designation, *Euphorbia lorifolia*.

EUPHORBIA ATROCOCCA **kokeeana** var. nov.—Folia pauciora, latiora (principalia plerumque 1.2–1.8 cm. lata), atro-viridia, venis lateralibus manifestis.

Specimens examined: *Otto Degener* 8094, Waimea Canyon near Kokee Camp, Isl. Kauai, June 30, 1926 (Herb. Degener, 2 sheets; Herb. Field Mus., 2 sheets; Herb. N.Y. Bot. Gard.); *Charles N. Forbes* 435K, Kaholuamanu, behind Waimea, Isl. Kauai, September, 1909 (Herb. Field Mus., 2 sheets); *Amos Arthur Heller* 2858, on Kaholuamanu, above Waimea, Isl. Kauai, Sept. 10–16, 1895 (Herb. Bishop Mus.); *idem* (*similiter*) 2858, *eodem loco*, Sept. 24–30, 1895 (Herb. Field Mus.); *idem* (*similiter*) 2858, *eodem loco*, Oct. 1–8, 1895 (Herb. Gray; Herb. Kew; Herb. Missouri Bot. Gard.; Herb. N.Y. Bot. Gard.; Herb. U.S. Nat.); *Joseph F. Rock*, Kaholuamanu, Isl. Kauai, October, 1911 (Herb. Field Mus.); *idem* 10099, below F. Gray's mountain house, Kaholuamanu, Isl. Kauai, October, 1911 (Herb. Bishop Mus.; Herb. Gray); *idem* 12933, Kaholuamanu, *eodem loco* (Herb. Bishop Mus.); *Dr. Carl Skottsberg* 1017, between Kokee and Mohihi, Isl. Kauai, Oct. 29, 1922 (type, Herb. Bishop Mus.).

On most specimens the apparently mature capsules are, as in the species proper, devoid of good seed. On two of the cited *Degener* specimens, however, a few ripe seeds were found. These were ovate-oblong in outline, truncate at base, obtuse at apex, acutely tetragonal with prominently carinate angles, reddish brown to brownish black, transversely scrobiculate, 1.8–2 mm. long.—Heller (Minnesota Bot. Studs. 1:844. 1897) described this variety as "a well

marked form, growing at an elevation of 4000 feet, near the edge of the woods."

EUPHORBIA ATROCOCCA **kilaueana** var. nov.—A specie ramis erectioribus elongatioribusque foliis tenuioribus eleganter spathulato-oblanceolatis apice saepius acutiusculis lamina 3–5.3 cm. longa differt. Inflorescentia ignota.

Specimens examined: *Abbé Urbain Faurie* 470, Kilauea, Isl. Kauai, January, 1910 (type, Herb. Brit. Mus.).

Coreocarpus johnstonii sp. nov.—Suffruticosa, e radice lignea erecta, ∓3 dm. alta, trichotome ramosissima ramis glabris vel parce hispidulis, lateralibus divaricatis et plerumque crassioribus, sicca non vel vix fragrans. Folia petiolo glabro vel interdum subhispidulo 3–8 mm. longo adjecto circ. 1–1.8 cm. longa, plerumque bipinnatisecta, segmentis ultimis crassiusculis lineari-oblongis vel subovatis subobtuse dentatis. Capitula numerosissima, cymose ad ramorum apices congregata, pansa ad anthesin ∓9 mm. lata et ∓6 mm. alta, pedicellis tenuibus vel tenuissimis minutissime 1–3-bracteolatis 1–4 cm. longis. Involucrum turbinatum, glabratum, bracteis paucis (plerumque 5 rarius usque ad 7), subaequalibus, ovatis, apice acutis, tantum circ. 3 mm. longis et quam disci floribus fere dimidio brevioribus. Flores ligulati circ. 3, albido-flavi; ligula oblongi, integri, +2.5 mm. longi. Achaenia exteriora corpore ipso 2.5–3 mm. longa et 1–1.3 mm. lata, alis plene in dentes nunc contiguos nunc separatos incisis, apice calvo; interiora angustiora, alarum dentibus saepe subremotioribus.

Specimens examined: *Ivan M. Johnston* 4293, common in dense low masses on a gravelly beach, San Pedro Bay, State of Sonora, Mexico, Jul. 7, 1921 (type, Herb. California Acad. Sciences).

I am indebted to MISS ALICE EASTWOOD, Curator of the Botanical Department, California Academy of Sciences, for the lending of this and other materials of *Coreocarpus* from the herbarium of that institution.

Coreocarpus sonoranus sp. nov.—Perennis, pallido-viridis, infra fruticosa divaricato-ramosaque supra herbacea adscendenti-ramosaque, sparsissime patenti-hispidula, ∓6 dm. alta, ramis tenuissimis elongatis superioribus subnudis. Folia bipinnatisecta petiolo tenuissimo 5–18 mm. longo, lamina 2–3.5 cm. longa, segmentis 1–2.5 (raro

-3) mm. latis saepius anguste oblongo-linearibus apicibus dentibusque subulatis.. Capitula laxe disposita (vix subcorymbosa), tenuissime pedunculata (pedunculis 1–5 cm. longis, basi subulato-bracteatis), pansa ad anthesin 1.4–1.8 cm. lata et 5–6 mm. alta, cum fructibus circ. 7 vel 8 mm. crassa et circ. 4 mm. alta. Involucrum campanulatum vel late subcylindricum, bracteis oblongo-ovatis apice saepe subacuminatis demum 3–4 mm. longis. Flores ligulati plerumque 5, rosacei (praecipue secundum venas), tubo sparsim hispidulo, ligula oblonga vel lineari-oblanceolata apice denticulata 5–8 mm. longa. Paleae lineares vel oblongae, apice saepius acutae, disci floribus superatae sed achaenia superantes. Disci flores aurantiaci circ. 2.8 mm. longi. Achaenia exteriora corpore ipso (nigro, ventraliter et rarissime dorsaliter papillato-hispidulo) circ. 3 mm. longa et 1–1.2 mm. lata, alis plene in dentes nunc contiguos nunc separatos subbrunneos sub 1 mm. longos incisis, apice calvo; interiora angustiora.

Specimens examined: *Marcus E. Jones* 23365, Guaymas, State of Sonora, Mexico, Jan. 26, 1927 (type, Herb. Pomona College: cotypes, Herb. Gray; Herb. Univ. California).

I am indebted to Dr. Philip A. Munz, Professor of Botany, Pomona College, for the lending of this and other materials of *Coreopsis* from the herbarium of that institution.

Coreopsis stillmanii jonesii var. nov.—Herba ∓2 dm. alta, basi valde ramosa. Capitula pansa ad anthesin 3.5–4 cm. lata et circ. 1 cm. alta. Involucrum glabratum, bracteis exterioribus 8–16, tenuissime linearibus superne usque ad apicem obtuso-truncatum sensim angustatis, 7–12 mm. longis, demum saepe patentibus vel subreflexis, quam interioribus oblongo-ovatis paulo brevioribus. Achaenia corpore (ipso nigro et circ. 1 mm. lato) glaberrima vel secundum costam medianam sparsim papillato-hispidula alibi interdum paucissimis setulis (nunc acribus nunc terminaliter capitatis) munita, 4–4.5 mm. longa et (alis stramineis inclusis) circ. 2 mm. latis.

Specimens examined: *Marcus E. Jones* 3361 *pro parte*, Pasadena, California, May 2, 1882 (type, Herb. Pomona College).

With some, this would pass as a distinct species. In *C. douglasii* (a sister species), however, the latitude of variations in number, size, and shape of exterior involucral bracts is found to embrace the ex-

tremes represented (correspondingly) by *C. stillmanii* and var. *jonesii*. What is more, in *C. douglasii* these variations appear so often transitional that not even a varietal segregation seems justified.

COREOPSIS BORIANIANA Schz. Bip. *ex* Schweinf., Verhandl. Zool.-Bot. Ges. Wein 18:684. 1868.—Recently, through the great kindness of DR. H. HUMBERT, Director of the Museum of Natural History of Paris, I have been lent the type of *Coreopsis chevalieri* O. Hoffm. & Muschl. (Bull. Soc. France 57, Mem. VIII, 118. 1910). This proves to be *C. borianiana* Schz. Bip. *ex* Schweinf.

Bidens brucei sp. nov.—Herba fruticosa, perennis, erecta, ∓ 1.8 m. alta, caulibus ramisque angulatis et non (nisi summam versus) pubescentibus. Folia opposita inferne in petiolos hispido-tomentulosos usque ad 1.5 cm. longos supra saepe marginatos abrupto-angustata, petiolo adjecto 6–11 cm. longa et 2–4.5 cm. lata, indivisa, lanceolato-oblonga vel saepius subrhomboideo-ovata, grosse serrata, apice subacuta, utrinque hispida. Capitula pauca, pedunculos subrobustos apicaliter folio-bracteatos terminantia, radiata, pansa ad anthesin circ. 7 cm. lata et circ. 1.5–1.8 cm. alta. Involucri hispidi bracteae 3–4-seriales, laxe adgregatae interdum patentes vel subreflexae subaequales, plus minusve oblongae, apice acutae, 1–2 cm. longae, intimae apicem versus angustatae. Flores ligulati 10–12, flavi, ligula oblongi vel lineares, apice parce denticulati, 3–4 cm. longi et 0.6–1.6 cm. lati. Paleae oblonge lineares, apice subacuto coloratae alibi stramineae 1.1–1.4 cm. longae. Achaenia late vel anguste oblongo-linearia, obcompressa, nigra, utraque facie circ. 8-sulculata, exalata, marginibus et facierum lineis perspicue erecteque papillato-setulosa, corpore 6–8 mm. longa et 1–2 mm. lata, apice erecte pauci-setosa et 2- (vel faciei ventralis costa mediana extensa saepe sub-3-)aristata aristis inferne robustis erecto-setosisque superne nudis tenuibusque ∓ 1 mm. longis.

Specimens examined: *Miss E. M. Bruce* 26, alt. 2200 ft., Ulugurue, Morogoro, Tanganyika Territory (German East Africa), Oct. 25, 1934 (type, Herb. Kew; *nom. vulg.*, m-luga); *B. D. Burtt* 4696, herb 3–4 ft. tall, on steep grass slopes, alt. 3000 ft., Uluguru Mts., above Morogoro, Tanganyika Territory, May 9, 1933 (Herb. Kew, 2 sheets).

BIDENS BRUCEI **pubescentior** var. nov.—A specie caulibus ramis-
que breviter patenti-hispidulis achaeniorum minorum (corpore 6–7
mm. longorum) apice numerose erecteque setoso aristis regulariter 2
tenuissimis ∓ 2 mm. longis non nisi base ipsa setosis differt.

Specimens examined: *G. B. Wallace* 294, herb. 6 ft. tall, alt. 3000
ft., Morogoro District, Tanganyika Territory, Feb. 16, 1932 (type,
Herb. Kew; *nom. vulg.*, luzasu).

This species stands between *Bidens crataegifolia* (O. Hoffm.)
Sherff and *B. coriacea* (O. Hoffm.) Sherff. I am indebted to SIR
ARTHUR W. HILL, Director, and MISS E. A. BRUCE, Assistant Bota-
nist, at the Royal Botanic Gardens of Kew, for the privilege of study-
ing the original specimens. Related to these are now seen to be two
hitherto enigmatic specimens collected some years ago by *Swynner-
ton* and named here:

BIDENS BRUCEI **swynnertonii** var. nov.—A var. *pubescentiore*
achaeniis corpore 7–9 mm. longis et 2–2.2 mm. latis floribus ligulatis
(tantum 8 pro singulo capitulo visis) forsan paucioribus differt.

Specimens examined: *C. F. M. Swynnerton* 859, Hiwaga, Tanga-
nyika Territory, April–June, 1921 (type, Herb. Brit. Mus.); *idem*
860, Kigobcra, Tanganyika Territory, May–June, 1921 (Herb. Brit.
Mus.).

BIDENS PILOSA var. RADIATA f. **dondiaefolia** (Less.) comb. nov.;
Bidens dondiaefolia Less., Linnaea **5**:155. 1830.

BIDENS PILOSA var. RADIATA f. **decumbens** (Greenm.) comb. nov.;
Bidens decumbens Greenm., Proc. Amer. Acad. **34**:576. 1899.

Bidens insolita sp. nov.—Verisimiliter perennis suberectaque;
caulibus gracilibus, angulatis, glabratis, non nisi ad summam ramo-
sis, ∓4 dm. altis Folia opposita, petiolata petiolis planis convexo-
concavisve sparsim setoso-ciliatis sub 2 cm. longis, petiolo adjecto
usque ad 1 dm. longa, bipinnatisecta, foliolis plerumque 5, saepe
(segmentis linearibus apicaliter acerrimis membranaceis glaberrimis
antrorsum spectantibus) subflabelliformibus. Capitula (4–8) sub-
corymbose disposita pedicellis tenuibus usque ad 8 cm. longis, radi-
ata, pansa ad anthesin circ. 3–3.5 cm. lata et 8–10 mm. alta. In-
volucri bracteae exteriores plerumque 8, lineares, extus glabratae,
marginibus albido-ciliatae, apice induratae et saepe acres, 4–5 mm.

longae, quam interiores ovato-oblongae apice pubescenti saepe abrupte angustatae breviores. Flores ligulati intense flavi, ∓1.5 cm. longi. Paleae lineares, superne angustatae, inferne interdum 1–3-dentatae dentibus erectis, 5–6 mm. longae. Flores tubulosi tenuissimi, corolla tubo brevi (circ. 1.5 mm.) adjecto demum circ. 5.5–6 mm. longa. Achaenia cuneato-linearia, subplana, brunneo-atra, utraque facie 4-sulcata, superne sparsim erecto-setulosa, corpore circ. 5–5.5 mm. longa et supra circ. 1 mm. lata, apice biaristata aristis flavidis retrorsum hamosis 2–3 mm. longis.

Specimens examined: *Howard Scott Gentry* 1971, meadow, Quicorichi, Rio Mayo, State of Chihuahua, Mexico, Oct. 7, 1935 (type, Herb. Field Mus.).

Bidens gentryi sp. nov.—Herba perennis, gracillima, circ. 1 m. alta; caulibus paucibus e unica radice, parce rectis sed non volubilibus, subteretibus, sulculatis, tantum circ. 1–1.3 mm. crassis. Folia opposita, petiolata petiolis tenuibus glabris plerumque 1–3 cm. longis, petiolo adjecto principalia 1–1.3 dm. longa, bipinnata vel tripinnatisecta; foliolis lateralibus primariis (imis tenuiter petiolulatis) 2 vel 3 jugis, membranaceissimis, glabratis vel margine sparsim setuloso-ciliatis, segmentis plus minusve lineari-oblongis vel ovato-lanceolatis terminali anguste attenuato; folioli terminalis segmento terminali angustissime lineari-acuminato usqụe ad 5.5 cm. longo. Capitula corymbose disposita (3 vel 4 ad caulis finem), pedunculata pedunculo tenui glabro ∓7 cm. longo, radiata, pansa ad anthesin circ. 3.8–4.3 cm. lata et ∓1.2 cm. alta. Involucri bracteae exteriores circ. 8, patentes vel subreflexae, lineares, apice acutae, glabratae, circ. 9–12 mm. longae; interiores oblongo-lanceolatae, non nisi apice pubescentes, breviores. Flores ligulati 7–9, flavi, ligula elliptico-oblanceolati, apice saepe 2- vel 3-denticulati, ∓2 cm. longi. Paleae superne sensim angustatae, apice obtusae, sub 1 cm. longae. Achaenia submatura linearia, subtetragono-obcompressa, brunneo-atra; corpore glaberrimo vel apicem versus sparsissime erecto-setuloso, sub 1 cm. longo et sub 1 mm. crasso, omnibus (4) faciebus 2-sulculatis; aristis abortivis vel 2, tenuibus, sub 3 mm. longis, suberectis, retrorsum hamosis hamis paucis albidis acerrimis.

Specimens examined: *Howard Scott Gentry* 1700, growing a meter high, stalks several from the base, pine slope in Upper Sonoran re-

gion, Pinal, Sierra Charuco, State of Sonora, Mexico, Sept. 9, 1935 (type, Herb. Field Mus.).

Intermediate between *Bidens reptans* var. *urbanii* (Greenm.) O. E. Schulz of southern Mexico, Guatemala, and the West Indies and *B. urophylla* Sherff of Brazil. From the former it differs in its larger capitula, longer exterior involucral bracts, eciliate achenes, etc. From the latter it can be told at once by its more compound leaves, larger and more numerous ligulate florets, etc.

The last two species of *Bidens* and the following variety of *Cosmos* were among materials very kindly submitted to me by DR. PAUL C. STANDLEY, Associate Curator of Botany at Field Museum of Natural History, Chicago.

COSMOS LINEARIFOLIUS **magnifolius** var. nov.—A specie foliis paucioribus majoribus principalibus 1–1.9 dm. longis et 6–10 mm. latis pedunculis usque ad 2.2 dm. longis differt.

Specimens examined: *Howard Scott Gentry* 1777, widely scattered, growing up along with grass, arid pine slopes, Arroyo Hondo, Sierra Charuco, State of Chihuahua, Mexico, Sept. 11, 1935 (type, Field Mus.).

MEGALODONTA BECKII **oregonensis** var. nov.—A specie foliis submersis inferioribus (1–1.6 cm. longis) quam superioribus minoribus ac minus decompositis differt.

Specimens examined: *Frederick V. Coville* and *Elmer I. Applegate* 42, in the marsh at Buck Lake, yellow-pine woods, Klamath County, Oregon, Jul. 24, 1897 (2 type sheets in Herb. U.S. Nat.; cotype, Herb. Gray).

Not to be confused with *M. remota* Greene, from Green Lake, near Seattle, Washington, which latter species seems, from its two sheets of original material examined (*Piper* 1114, Herb. Gray), to represent *M. beckii* rather than my variety.

CHICAGO NORMAL COLLEGE

TUSSOCK MEADOWS IN SOUTHEASTERN WISCONSIN

DAVID F. COSTELLO

(WITH NINE FIGURES)

Introduction

Throughout eastern Wisconsin, and extending eastward to the Atlantic seaboard, is a type of sedge meadow which seems to have been little studied and scarcely mentioned in the literature. It is characterized by numerous tussocks (figs. 1, 3), formed by sedges with the caespitose habit, which reach a height of 1–3 feet and which are separated by passages 1–4 feet wide. In bluegrass pastures the remnants of these tussocks are frequently represented by grass covered mounds a few inches in height. They almost invariably attract the attention of the traveler entering the region for the first time, but are taken for granted by the inhabitants, who usually explain their presence as being due to the trampling of livestock.

Although the floristic composition and the gross environment of these tussock meadows are fairly well known, ecological studies concerning the tussock habit, the floristic structure of the tussock association, and the factors which accompany and influence the initiation, development to maturity, and subsidence of these communities have been neglected. DRUDE (2) has briefly characterized similar associations in Hungary and has mentioned the gradual replacement of tussock meadows by grassland. WARMING and GRAEBNER (11) mention the formation of tussocks by *Carex paniculata* and *C. stricta* in Europe, and discuss at length the tussock vegetation of the subantarctic islands. They state that "tussock" signifies in general a vegetation of thick, high, bushlike tufts of grasses or grasslike plants. These tufts, composed principally of leaves, surmount compact pedestals which are formed from dead stems and leaf débris and which are ramified by numerous rhizomes and roots. The old pedestals frequently serve as substrata for other plants. NICHOLS

(6) states that the tussock sedge, *Carex stricta*, is "by far the commonest, and ecologically the most important, sedge of Connecticut swamps." He mentions its place in the usual swamp succession, lists a number of species characteristic of the sedge stage, calls attention to the composite habitat afforded by tussock meadows, and notes that the duration of the sedge stage may be long or short. In Wisconsin, STOUT (9) has studied the tussocks formed by *Carex stricta* in

FIG. 1.—*Carex stricta* tussock showing pedestal and tuft of leaves; *Ranunculus delphinifolius* on the ground.

connection with the other vegetation in a wild hay meadow. He gives statistical data concerning the frequence and abundance of the various species, but his work appears to deal with a mixed community rather than with a relatively pure stand of the tussock sedge. He describes the stooling habit of this sedge, discusses briefly its growth form and adaptations, and points out that the exclusiveness of *Carex stricta* is a problem that needs further study. In the introduction to his paper he includes a summary of the literature dealing with the geographic distribution of meadows and the classi-

fication of low moist formations. Although his paper does not deal
primarily with the ecology of *Carex stricta*, it contains the best exist-
ing discussion of the species that has been written to date.

The present paper deals with the ecology of the tussock sedge, the
structure of the tussock association, the place of the association in
succession, and the environmental and biotic factors which accom-
pany and influence the association throughout its life history.

The area included in this study comprises most of Ozaukee,
Washington, Waukesha, Milwaukee, and Racine counties in Wis-
consin. General observations were made of most of the meadow as-
sociations in this region within a radius of 35 miles from the city of
Milwaukee. An area was selected for detailed study near the south-
ern boundary of Ozaukee County, about 2 miles south of Cedarburg,
on the west side of State Highway 57. The strip of meadow studied
is somewhat over a quarter of a mile long and an eighth of a mile
wide. The east side is low and moist, and consists principally of
tussock meadow through which a small stream meanders. The west
side rises gradually toward the top of a high ridge. Half way up this
ridge, where the soil is kept moist by numerous springs, the tussock
meadow again appears. Three-fourths of a mile northwest of this
area an ephemeral pond with its surrounding tussock meadows was
selected for further detailed study. Both of these areas are typical
of scores of others that may be found within the same region.

Observations, beginning in 1927, were made over a period of six
years. Detailed work was done in the field during the summers of
1929, 1932, and 1933. Studies of the growth habit of the tussock
sedge were made during the winter as well as the summer.

Topographic and physiographic features

The areas considered in this paper all lie within the glaciated
region of eastern Wisconsin. Consequently the topography is youth-
ful, the relief is moderate, and the country is gently rolling. The
topography is controlled by the Niagara cuesta (5), which is also
spoken of as the eastern upland. The upland is underlain by Niagara
limestone which dips eastward toward Lake Michigan. Covering the
rock is a mantle of glacial drift of variable thickness, consisting
principally of till, sand, gravel, and clay. In Waukesha and Wash-

ington counties the Kettle Moraine, an interlobate deposit, is characterized by greater irregularity and by pits or undrained depressions.

Temporary lakes and swampy tracts are common throughout the district. Many of these swampy areas are the result of filling of lakes; others are the result of seepage and poor drainage. According to WHITBECK (14), "Between twelve and fifteen per cent of this area is covered with lakes, swamps, or land that has been swampy." He also states that "not all of the present swamps were former lakes; probably the majority of them have been swamps from their beginnings." It is in these areas and in the drained depressions of the Kettle Moraine that tussock meadows have developed.

Methods

Floristic composition was studied by means of meter quadrats. Quadrats of this size were deemed necessary because of the uneven distribution of the vegetation. Transition areas and tension zones were studied by means of belt transects 0.5 and 1 m. wide. Immature plants were marked by stakes and later identified when flowers or fruit appeared. Doubtful plants were carefully pressed and later checked with authentic herbarium specimens. The presence and number of individuals and the coverage and height of each species within the quadrats were recorded. In order to facilitate counting, the plants were clipped from each quadrat. The culms of grasses and sedges were counted as individuals. Small plants such as *Eleocharis palustris* and *E. acicularis* were not counted but were rated for coverage and height.

In the study of the subterranean plant organs, trenches were dug to a depth greater than that of the deepest roots and the soil was then dissected away from the underground parts with an icepick or other sharp instrument. For more detailed studies of the roots in tussocks the whole pedestal was dug up and transported to the laboratory for dissection. Counts of the roots were made in winter when the frozen pedestal could easily be chopped off with an ax. For purposes of propagation, pieces of tussocks containing both roots and buds were brought into the greenhouse in winter and planted in soil or in water cultures.

Soil samples for moisture determination were taken weekly at depths of 6, 12, and 18 inches. The percentage of moisture was calculated on the basis of the dry weight of the soil.

Evaporation readings were taken from standardized porous porcelain atmometers. The usual precautions concerning rain absorption, the entrance of rain or dew into the bottles, and standardization of the bulbs were observed.

The level of the water table was determined throughout the growing season by weekly readings taken from numerous wells in typical parts of the area. These wells were plugged to reduce evaporation and to prevent seepage from rains or the accumulation of débris from the surface. At frequent intervals they were cleaned and then left undisturbed for at least 24 hours before a reading was taken.

The hydrogen ion concentration of the soil was determined in the field by means of the Morgan soil tester. These readings were checked in the laboratory on dried soil samples with the quinhydrone apparatus.

Vegetation of tussock meadow

Well developed tussock meadows are always dominated by a single species, *Carex stricta*. This plant frequently comprises more than 90 per cent of the vegetation and controls the physiognomy both in summer and in winter. In summer the deep green leaves obscure the pedestals beneath, but the presence of the tussocks is made apparent by the undulating nature of the sedge cover (fig. 2). In winter the hummocky nature of the area is most apparent (fig. 3). The dead leaves and culms, beaten down by wind and rain, form a close thatch about each pedestal.

It is only during the growing season that secondary species are at all conspicuous in the tussock association. In early spring these are apparent because of their showy flowers and the delayed development of the tussock sedge. The summer and autumn species are visible only because they surmount the general level of the meadow. Other species remain secluded beneath the sedge cover and are found only by pushing aside the tufts of leaves that grow from the tussocks.

ECOLOGY OF TUSSOCK SEDGE

INITIATION AND GROWTH OF TUSSOCKS.—New tussocks are initiated by rhizomes which grow down over the edge of the pedestal in the protection of the thatch of dead leaves, when the tussock is in the water, or which grow horizontally just beneath the soil when
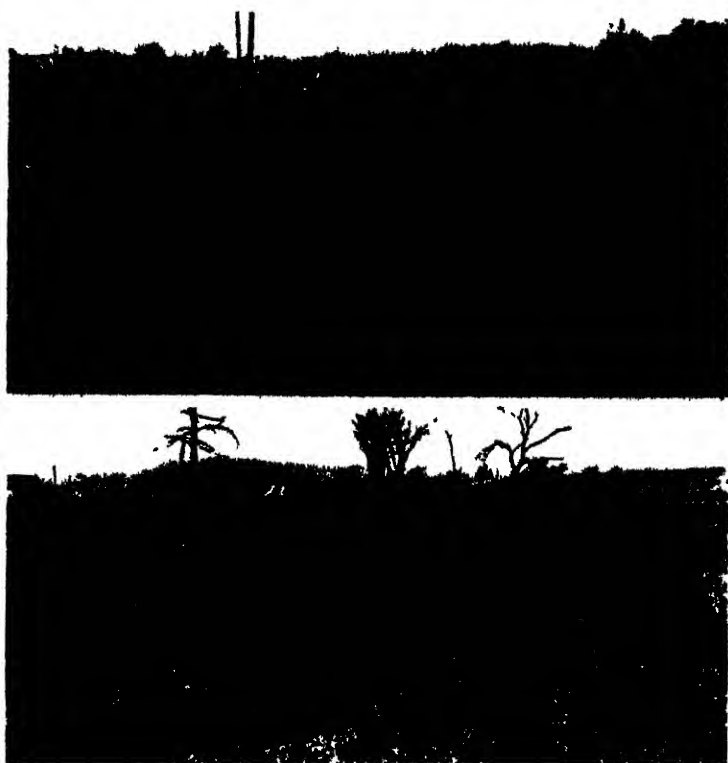
FIGS. 2, 3.—Fig. 2 (above), summer aspect of *Carex stricta* association; *Eupatorium* in foreground, *Typha* in background. Fig. 3 (below), well developed tussock association; water table about 4 inches below ground level.

the base of the tussock is a mere rounded hillock in drier situations. The pointed terminal buds of these rhizomes turn upward, producing a tuft of leaves, while roots develop from the base of the bud just beneath the soil. Under the most favorable conditions new tussocks are initiated in water that varies in depth from 6 to 12 inches. In a comparatively short time the leaves and culms appear above the

surface and act as a trap for floating débris, initiating the tussock almost as soon as the leaves appear.

New leaves appearing at the base of the young tuft throughout the growing season result in a well developed clump by the end of summer. Within a few years a pedestal consisting of dried culms, woody stems, old rhizomes, roots, soil, and vegetable matter is built up. Eventually the pedestals rise above the surface of the water (fig. 4), or during dry seasons they stand high and dry (fig. 1). In time

FIG. 4.—*Carex stricta* tussocks invading a pond

the mass of material which supports the clump of leaves becomes a network of fine fibrous roots which grow from the bases of the culms or develop as branches of larger wirelike roots that grow downward into the muck (fig. 9). From the crown of dead leaves above, long pointed buds grow out to produce new foliage each year.

If undisturbed, a tussock for several seasons may consist of a single plant. The culms and rootstocks remain so closely attached that they are dissected with difficulty. Eventually decay of the old woody parts below the surface may separate the stems, with the result that the tussock becomes a mass of interwoven but distinct individuals.

The age to which tussocks may attain is problematical. There is little doubt that under normal conditions they grow and develop for many years. Tussocks initiated in 1928 at the station south of

Cedarburg were 4 or 5 inches in diameter in 1934, but obviously these tussocks were then immature. Occasionally a vertical section through a tussock will show indications of layers of leaf bases and old stems that are deposited yearly. Calculations made on this basis would indicate an age of 40–60 years for some of the largest tussocks. Disregarding their destruction by violent means, by flooding, or by changes in the habitat, there is reason to believe that they may persist for half a century at least.

The rate of growth of larger tussocks is slow. Specimens marked for special study in permanent quadrats have shown almost no change in height or diameter over a period of 6 years. The maximum size to which they may ultimately attain depends upon the conditions under which the sedge grows. At the edges of ponds pedestals 2 feet in height with a diameter of 18 inches are not uncommon. From this size they grade down to small rounded mounds 2 inches in height and 6 inches in diameter.

The percentage of area covered by the bases of tussocks and the number of tussocks per unit area in different habitats are shown in table I. No correlation can be drawn between the percentage of area covered and the size of the tussock. The number of stools per unit area depends upon a great many factors, including the stage of development of the association, the degree of soil saturation, artificial drainage, annual fires, and grazing. In general, the larger the stools the smaller will be their number in a given area.

VEGETATIVE STRUCTURES OF TUSSOCK SEDGE.—The ability of *Carex stricta* to occupy certain areas in almost pure stands depends to a considerable extent upon the habits of the vegetative structures. Plants which at first appear to compete with the tussock sedge may be shown, by an investigation of root and rhizome habits, and of stratification above ground, to be adapted to different environments. The vegetative habits of *C. stricta* seem to offer at least a partial solution to this problem.

ROOT SYSTEM.—Two types of roots are present: long, cylindrical, wirelike soil roots which grow straight down into the substratum and slender, tortuous, much branched, fibrous roots which permeate the earthy pedestal of the tussock. The soil roots arise from the sides of the culms or from the short compressed internodal regions

of the woody stem which lie just beneath the surface of the tussock (fig. 9). They are of rather uniform diameter, varying from 2 mm. in thickness at the point of origin to 0.5 mm. a short distance back from the tip. They range in length from 1.5 to 7 dm., depending upon the nature of the substratum, compactness of the soil, water table, and moisture content of the soil. Within the pedestal, especially when aeration is good, they may branch freely, with the result that a matted spongy mass is formed. Below the general soil level branching is less pronounced. This is especially apparent in winter when

TABLE I

COMPARISON OF SIZE AND NUMBER OF TUSSOCKS, AND PER-
CENTAGE OF AREA COVERED BY THEIR BASES

HABITAT	AV. DIAMETER OF TUSSOCK (DM.)	NO. OF DIAM-ETER QUADRATS	AV. NO. OF TUSSOCKS PER SQ. METER	PERCENTAGE AREA COVERED BY TUSSOCK BASES
Permanent pond...........	3 0	64	1.5	10.6
Intermittent pond.........	3.2	43	2.3	18.5
Morainic depression........	3.5	55	1.9	18.2
Floodplain...........	3.0	80	3.6	25 4
Springy hillside............	3 1	35	4.1	10.9
Margin of stream..........	4.5	32	1.6	15.9
Pond margin..............	3.6	28	4.2	42.7
Floodplain (burned)........	1.5	40	5.0	8.8
Swale....................	5.0	100	0 9	17.6

the frozen hummock is chopped or broken off; the soil roots then project from the exposed surface like so many stiff wires.

The number of vertical soil roots within the pedestal is considerable. A cross section of a pedestal 1.5 dm. in diameter will show from 250 to 400 roots; larger tussocks will show a correspondingly greater number. The largest tussock examined was slightly over 8 dm. in diameter and showed approximately 3200 roots.

The depth to which the soil roots will penetrate is determined to a considerable extent by the ground-water level. In areas where the water stands continuously above the soil the root system is shallow. If the water table is fluctuating the roots may penetrate to the lowest level of the water table, a distance seldom more than 24 inches below the soil surface.

Root hairs are produced to a limited extent by the periodically submerged soil roots. If the soil is compact and clayey they may be entirely absent. Root hairs are produced in great abundance by soil roots, however, which frequently grow down the sides of the pedestal or which become exposed to the air by the lowering of the water in ponds. A thick feltlike covering is formed by the numerous hairs, which gradually lignify and turn brown. In the greenhouse they may be produced on plants grown in water culture by gradually lowering the water level, or by growing the plants in well aerated soil which is watered sparingly. Whether or not these root hairs possess any power of absorbing moisture from the air has not been determined.

RHIZOME SYSTEM.—Vegetative propagation of *Carex stricta* is accomplished by means of rhizomes. The rootstocks arise from the bases of the culms, and as STOUT (9) has pointed out, are of two types: the ascending and the creeping.

The terminal buds of the ascending rhizomes immediately give rise to new leafy shoots and thus increase the height and lateral spread of the tussock. The creeping rhizomes extend a few inches beneath the soil until they reach a length of a foot or more. The terminal bud may then turn upward to produce a shoot and initiate a tussock. Frequently the rhizomes of tall tussocks are bent over the edge of the pedestal, half concealed in the thatch of dead leaves, their long pointed buds directed toward the ground (fig. 9). Occasionally they grow downward through the mass of roots and then horizontally a short distance below the surface of the soil.

Carex stricta is not so strongly stoloniferous as *C. strictior*, which grows in somewhat similar situations and occasionally with the tussock sedge. *C. strictior*, however, does not produce distinctive tussocks but forms large flat clumps. Although the two species are sometimes distinguished with difficulty in herbaria, there can be no doubt of their identity in the field. MACKENZIE (4) has pointed out the taxonomic features of these two sedges, including the nature of the stolons.

While the ascending stolons provide both for leaf and flower production, it is apparent that the tussock sedge depends almost exclusively upon vegetative growth for its maintenance. Examination

of thousands of tussocks over a period of more than six years in the
Milwaukee region has yielded comparatively few fruiting specimens.
Seedlings of *Carex stricta* were never found during this period.

The leaves form a close fascicle as they arise from the ascending
stolon; hence in the quadrat studies leaf clusters or culms were
counted rather than individual leaves. A tussock 10 inches in
diameter will ordinarily show from 100 to 150 clusters. When fully
mature the leaves form a dense cover, shading even the open spaces
between the tussocks. In early summer the aspect is that of a
luxuriant meadow, all evidence of the uneven nature of the ground
frequently being obscured.

ECOLOGICAL ANATOMY.—The leaves of *Carex stricta* present a
comparatively small area of exposed surface. Although they vary in
length from 4 dm. to more than 1 m., they are seldom more than
7 or 8 mm. wide and are usually dried at the tips. When subjected
to drought they fold upward on the midrib, but the blades are stiff
enough to resist twisting by the wind.

In cross section the leaf is seen to consist of a series of parallel
bundles separated by compartments of chlorenchyma (fig. 5). The
bundles are surrounded by sclerenchymatous sheaths which are in
turn surrounded by non-chlorophyll bearing parenchymatous cells.
The chlorenchyma usually surrounds a group of non-chlorophyll
bearing cells. In older leaves the latter may break down to form
lacunae. The upper and lower epidermis possesses a cuticle of
medium thickness. Bulliform or motor cells occur in the groove of
the ventral (adaxial) side of the leaf. They provide for the folding
of the leaf by their collapse during periods of desiccation.

The leaves of *Carex stricta* are well adapted to withstand high
evaporation and the periods of summer drought to which they are
sometimes subjected, although they do not show the pronounced
xeromorphic structure which is characteristic of some of the upland
species of grasses (13). The persistence of the tussock sedge in dry,
well grazed pastures may be explained in part by its semi-xero-
morphic leaf structure.

The roots and rhizomes show both hydromorphic and xeromorphic
structure. HAYDEN (3) has pointed out that the primary indicators
of water relations in underground organs are the relative proportions
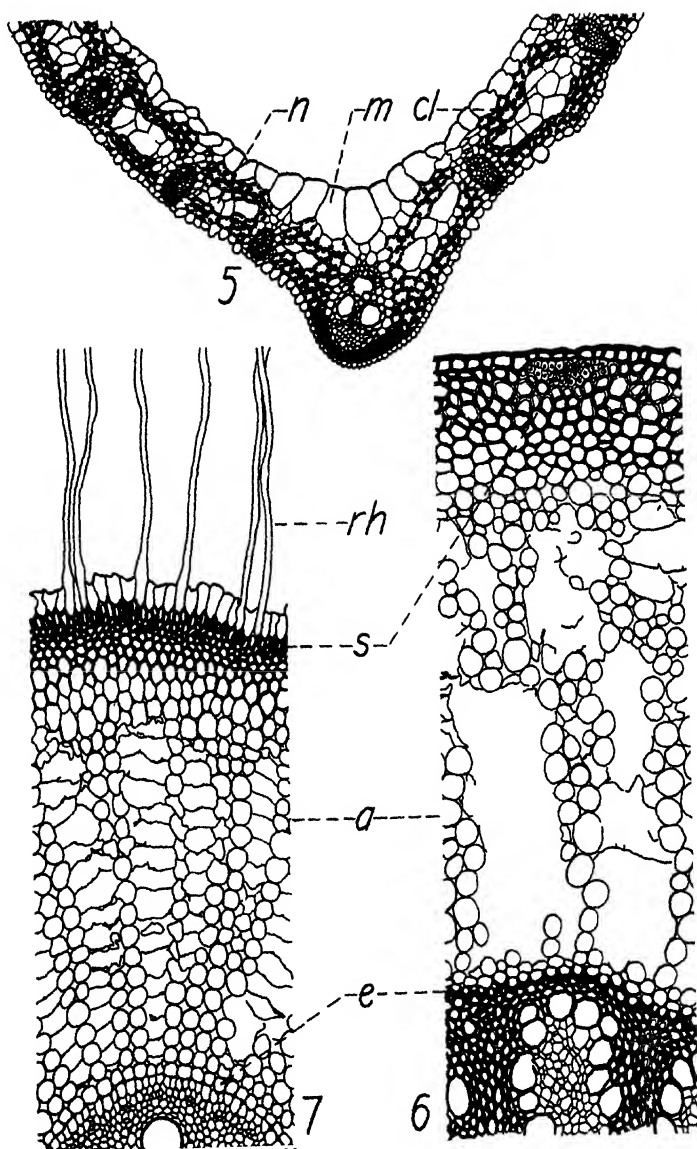
FIGS. 5–7.—Transverse sections of leaf, rhizome, and root of *Carex stricta* showing ecological anatomy: fig. 5, transection of leaf (*cl*, chlorenchyma; *m*, motor cells; *n*, non-chlorophyll bearing cells); fig. 6, transection of mature rhizome; fig. 7, transection of young root (*e*, endodermis; *a*, aerenchyma; *s*, sclerenchyma; *rh*, root hairs).

of parenchyma, mechanical tissue, and conductive tissue. Xeric structure is indicated by the prominence of the latter two, and the reverse condition is indicated by the first.

In the rootstock (fig. 6) the cortical tissue occupies two-thirds of the radius of the rhizome. The thick walled epidermis surrounds a sclerenchyma layer which is six to eight cells in thickness. The zone of aerenchyma is pronounced and is characterized by large lacunae. A prominent endodermis with thickened radial and inner tangential walls is present. The stele is a close fascicle of numerous bundles, each surrounded by a compact sheath, inclosing a small pith cylinder of thin walled aerenchyma.

In the root (fig. 7) the cortical area represents from two-thirds to three-fourths the diameter of the entire structure. Numerous lignified root hairs may or may not be present. The sclerenchyma layer beneath varies from five to eight cells in thickness. Aerenchyma is even more pronounced here than in the rhizome. The radial and inner tangential walls of the endodermis are strongly thickened. The stele is composed of a single row of bundles surrounding a pith cylinder of lignified cells.

The prominence of mechanical tissue in both the root and the rhizome would seem to indicate xerophytism, while the abundance of aerenchyma indicates hydric tendencies. This condition, however, does not appear to be anomalous when the water relations of *Carex stricta* are considered. As a rule both roots and rhizomes are exposed to the atmosphere for a portion of each growing season. Adequate protection from desiccation is afforded by a sclerenchyma layer in the outer part of the cortex. At other times the root and rhizome systems are submerged. The utility of an aerating system then becomes apparent. The presence of additional mechanical tissue in the stele explains to some extent the difficulty with which tussocks are uprooted or toppled over, either by artificial means or as the result of natural forces.

TUSSOCK ASSOCIATION

The vegetation of the tussock meadow is almost exclusively herbaceous. *Carex stricta* is the dominant of the association. Other sedges occur, particularly *C. bebbii, C. hystericina, C. vulpinoidea,*

and *C. lanuginosa*, but never in sufficient abundance to change markedly the floristic composition of the area. When present they are generally limited to the mesic side of the association. The more mesophytic grasses, *Calamagrostis canadensis*, *Glyceria nervata*, *Bromus ciliatus*, *Agrostis alba*, and *Phragmites communis*, occasionally change the appearance of the association late in the season owing to the height of their culms, which overtop the other species, or owing to contrast in the color of their foliage. Few species deserve to be designated as constants. *Asclepias incarnata*, *Lycopus americanus*, and *Equisetum arvense* appear in a high percentage of the stands but they may be totally absent. In late summer and early autumn *Eupatorium maculatum*, *E. perfoliatum*, *Solidago* spp., *Aster* spp., *Stachys tenuifolia*, and *Bidens frondosa* may be found in stands of considerable extent. Some of the conspicuous but ecologically unimportant species which occasionally appear are *Iris virginica*, *Symplocarpus foetidus*, *Caltha palustris*, *Lilium michiganense*, *Hemerocallis fulva*, *Spiraea alba*, and *Lobelia siphilitica*.

The composite nature of the habitat has been pointed out by NICHOLS (6). He mentions a few of the hydrophytic species which are able to grow in the moist substratum between the tussocks and a number of plants which are able to thrive in the drier substratum of the tussock itself. Shade tolerant species are especially characteristic of the inter-tussock vegetation. In general, species which propagate themselves mainly by vegetative means are found in the spaces between the tussocks. Typical species are *Equisetum arvense*, *Impatiens biflora*, *Marchantia polymorpha*, *Conocephalum conicum*, and *Typha latifolia*. Species which spread readily by seed commonly grow from the tops of the tussocks. *Bidens frondosa* is probably the best example, especially on tussocks growing in ponds. In early summer its seedlings may be found in the crowns of the tussocks. As the season progresses the tap roots grow down the sides of the pedestals, apparently keeping pace with the lowering water table. In well grazed pastures where *Carex stricta* persists only as a relict, *Verbena hastata* is almost invariably present in the crowns of the tussocks.

Woody forms are represented by various willows, especially *Salix discolor*, *S. petiolaris*, and *S. interior*. Occasionally *Spiraea alba*,

Cornus stolonifera, and *Rhamnus alnifolia* appear as isolated individuals in the meadow. More commonly the willows appear *en masse* at the margin of the association, where they represent the stage in succession that is to follow.

A statistical analysis of four stands is shown in table II. Stand no. 1 borders a small pond; it is about 3 acres in extent. Stand no. 2 covers the floodplain of a small stream, the ground sloping gradually upward on either side of the stream; it covers an area of approximately 15 acres. Stand no. 3 lies in a drained depression of the Kettle Moraine; it has an area of 4 acres. Stand no. 4 lies on an east facing slope characterized by seepage and underground springs; it occupies about three-fourths of an acre.

In table II the numbers or symbols in the columns have the following significance: constancy is indicated by the number preceding each period; vegetational cover is indicated by the number following. Species of infrequent occurrence are represented by a plus sign. When the species is absent from the stand this is indicated by a minus sign. Presence and cover are expressed in five degrees as follows (1):

Presence:

  1, present in less than $\frac{1}{5}$ of quadrats studied
  2, present in $\frac{1}{5}$ to $\frac{2}{5}$ of quadrats studied
  3, present in $\frac{2}{5}$ to $\frac{3}{5}$ of quadrats studied
  4, present in $\frac{3}{5}$ to $\frac{4}{5}$ of quadrats studied
  5, present in $\frac{4}{5}$ to $\frac{5}{5}$ of quadrats studied

Cover:

  1, covering feeble, less than $\frac{1}{20}$ of area
  2, covering $\frac{1}{20}$ to $\frac{1}{4}$ of area
  3, covering $\frac{1}{4}$ to $\frac{1}{2}$ of area
  4, covering $\frac{1}{2}$ to $\frac{3}{4}$ of area
  5, covering more than $\frac{3}{4}$ of area

COMPETITION AND ADAPTATION WITHIN THE ASSOCIATION.—A plant association may be more than a collection of species adapted in the same or in different fashion to similar conditions of habitat (9). It may include species which are not adapted and species which are so aggressive or capable of occupying the area that their presence

TABLE II

TUSSOCK ASSOCIATIONS

	1	2	3	4
NUMBER OF STAND	1	2	3	4
SIZE OF QUADRAT	1 SQ. M.	1 SQ. M.	1 SQ. M.	1 SQ. M.
QUADRATS SCORED	12	20	18	24
VEGETATION COVER %	70	100	100	100
TYPE OF HABITAT	BORDER OF POND	FLOOD-PLAIN	DEPRES-SION	HILLSIDE (SPRINGS)
Carex stricta	5 . 3	5 . 5	5 . 5	5 . 5
Solidago spp.	1 . 1	3 . 2	3 . 1	1 . 1
Equisetum arvense	+	2 . 1	3 . 1	—
Impatiens biflora	2 . 1	1 . 1	1 . 1	1 . 1
Eupatorium maculatum	+	2 . 2	1 . 1	+
E. perfoliatum	+	2 . 2	+	+
Asclepias incarnata	1 . 1	2 . 1	+	—
Bidens frondosa	3 . 2	—	—	—
Galium asprellum	—	3 . 2	—	—
Typha latifolia	—	1 . 1	—	—
Salix petiolaris	1 . 1	1 . 1	— .	—
S. interior	1 . 1	—	—	—
S. bebbiana	1 . 1	—	—	—
Caltha palustris	1 . 1	+	+	—
Iris virginica	1 . 1	—	—	—
Scirpus palustris	1 . 1	—	—	—
Rumex verticillatus	+	+	1 . 1	—
Stachys tenuifolia	—	+	2 . 2	—
Ambrosia artaemisiifolia	—	—	1 . 1	—
Viola sp.	—	1 . 1	—	—
Calamagrostis canadensis	—	—	1 . 1	—
Marchantia polymorpha	—	1 . 2	—	—
Conocephalum conicum	—	—	—	1 . 1
Barbula unguiculata	—	1 . 1	—	—
Symplocarpus foetidus	—	—	—	1 . 3
Hemerocallis fulva	—	+	—	—
Lilium michiganense	—	+	—	—
Smilacina stellata	—	—	—	1 . 1
Carex lanuginosa	—	1 . 1	—	—
Thalictrum dasycarpum	—	1 . 1	—	—
Spiraea alba	—	+	—	—
Verbena hastata	—	—	—	1 . 1
Campanula aparinoides	—	1 . 1	—	—
Aster novae-angliae	—	1 . 1	—	—
Lycopus americanus	—	2 . 1	1 . 1	—
Carex retrorsa	+	—	—	—
Carex spp.	+	+	+	+
Lysimachia thyrsiflora	+	—	—	—
Carex riparia	1 . 1	—	—	. —
Trifolium procumbens	—	—	+	—
Solanum dulcamara	—	—	—	+
Chelone glabra	—	+	—	—
Lobelia siphilitica	—	—	—	+
Helianthus grosseserratus	—	+	—	—

is detrimental to the characteristic species. Sherff (8) has empha-
sized two types of relationships which may exist within a com-
munity: the complementary, in which the plant organs grow and
vegetate at different levels; and the competitive, in which the plant
members occupy the same stratum. These relationships will be con-
sidered now from the standpoint of the aerial and of the subter-
ranean plant organs.

Aerial relationships.—Among the complementary species of
the association are those plants which show a marked seasonal adap-
tation. An examination of the tussock meadow in early spring shows
a number of species which flower and fruit long before the dense
growth of sedges has formed a closed cover. There are many shal-
low-rooting species present which become inconspicuous or disappear
entirely with the advent of summer. All of these grow in the spaces
between the tussocks where there is an abundance of moisture and
light during the early growing season. By June 15 they are usually
overtopped by the sedges.

In late summer and early autumn a considerable number of
species appear which exceed the general meadow cover in height but
which may be still considered mostly complementary in their aerial
relationships. The period of flowering and fruiting comes after the
typical meadow vegetation has ceased to grow vigorously or has
even become dry. *Eupatorium purpureum*, *Solidago serotina*, *He-
lenium autumnale*, and *Aster novae-angliae* may be placed in this
group.

Stratification is not especially marked in the tussock meadow,
although a number of species present show a definite light or
moisture requirement. Data obtained from atmometer studies (see
later paragraphs) in these meadows conform to the findings of Yapp
(15) that the evaporation rate rapidly increases from the ground
upward. There is little doubt that the presence of mosses and liver-
worts at the lowest level may be correlated with the abundance of
moisture, the higher humidity, and the dense shade. Among the
species which grow in the shade of the tussock sedge are *Impatiens
biflora*, *Ambrosia artemisiifolia*, *Equisetum arvense*, *Dryopteris the-
lypteris*, *Trifolium procumbens*, *Pilea pumila*, and seedlings of

Xanthium sp. Owing to their relatively weak growth, none of these may be considered as aerial competitors of the sedge.

Among the competitive species of greatest importance are those which grow concurrently with the dominant vegetation. Most of the sedges and grasses of the association belong to this group. Of these, *Calamagrostis canadensis* is probably the most important. In the drier portions of a tussock meadow the culms of this grass may occasionally equal or exceed in number those of *Carex stricta*. In late summer the grass gains the ascendancy over the sedge and may even obscure the latter in dense stands. In the moister areas, however, *Calamagrostis* loses its aggressiveness and appears only in scattered bunches, if it is present at all. Of lesser importance are the sedges, *Carex vulpinoidea, C. hystericina, C. lanuginosa*, and *C. stipata*, and the grasses *Phragmites communis, Bromus ciliatus, Spartina pectinata, Leersia oryzoides*, and *Glyceria nervata*. Other species such as *Scirpus atrovirens* and *S. lineatus* are of such infrequent occurrence that they are of little importance as competitors.

A number of species which may be considered as strongly competitive are those which commonly grow on the tops of the tussocks. DRUDE (2) states in his description of *Carex stricta* meadows in Hungary that a motley array of plants establish themselves on the tops of the tussocks even when they are still in ponds. However, he mentions specifically only the thistles and orchids. NICHOLS (6) mentions a number of plants that grow on the tussocks in Connecticut swamps. In general, plants which reproduce by seeds appear to be the most frequent invaders of the tussocks themselves. At best these intruders find themselves in uncongenial surroundings. They have to contend with the overwhelming numbers of sedge leaves; their roots must penetrate a tangled substratum of dead culms, rhizomes, and matted rootlets; and not infrequently the top of the tussock dries out before the young seedlings have established themselves.

One of the most successful of these invaders is *Bidens frondosa*. It begins its growth in the middle of summer and attains its greatest luxuriance long after the sedges have reached their maturity. It reaches its greatest abundance in areas which are shaded by taller

species, particularly the willows. Its presence in such situations is
a good indication that the tussock association is disappearing.

SUBTERRANEAN RELATIONSHIPS.—An examination of the root sys-
tem of *Carex stricta* reveals the fact that the greatest competition
in the tussock meadow is not above but below the surface of the soil.
It explains to a certain extent the ability of the tussock sedge to
occupy the habitat almost to the exclusion of everything else. Aided
by its aerating system, the sedge permeates the saturated soil with
roots and rhizomes. The interrelationships of the subterranean
organs of the tussock sedge with other species and their possible sig-
nificance will be considered now.

SAGITTARIA.—This plant, particularly in ponds being invaded by
Carex stricta, frequently forms a border on the hydric side of the
tussock association. It almost never mingles with the tussocks. The
transition from the *Sagittaria* zone to tussock meadow is abrupt.
SHERFF (8) has pointed out that the habit of growth of *Sagittaria*
does not favor a compact grouping of individuals, since the rhizomes
are not of the mat producing type. It can be invaded easily by *C.
stricta*. On the other hand, although rhizomes of *Sagittaria* grow to
a depth of 15 cm., they cannot grow beneath the deeper root system
of the sedge. Thus it appears that the sedge is limited in its advance
on the pond not by the zone of *Sagittaria* but by its own ability to
grow in deep water.

SPARGANIUM AND TYPHA.—These frequently border the tussock
association on the hydric side, especially along streams and occasion-
ally in ponds. Relict stands of *Typha* are sometimes found in the
midst of an otherwise well developed tussock meadow. Root and
rhizome competition is always marked between these species. The
densely matting rhizomes of both genera are instrumental in main-
taining almost pure stands, once they have become established. In
addition, their compact habit of growth excludes *Carex stricta*, until
the building up of soil and the consequent lowering of the water table
so modify habitat conditions that the aggressiveness of *Sparganium*
and *Typha* disappears.

CAREX RIPARIA.—Dense stands of this rank growing sedge are
sometimes found in the tussock association, although they are not
a part of it. These stands may occur on the hydric side, the mesic

side (as islands within the association), or as broad bands running completely through the association. Superficial examination discloses no very great variation in the habitat. The area occupied by *Carex riparia* apparently should support a luxuriant tussock meadow. The explanation rests upon a number of factors.

The rootstocks of *C. riparia* are very coarse and are capable of rapid growth. From the base of the culm they grow downward, and at a depth varying from 5 to 20 cm. they assume a horizontal direction for a distance of 10 to 50 cm.; the tips then grow vertically to give rise to a new plant at the surface of the ground. Roots are produced both from the base of the culm and from the nodes of the rootstock. Thus the entire soil layer to a depth of 15–20 cm. is permeated with a coarse mat of subterranean organs. Growth of the rhizomes is very rapid in the loose spongy soil.

The areas occupied by *C. riparia* are almost invariably flooded by débris and soil washed from surrounding uplands. The accumulated soil may amount to 6 inches or more within a single season. Owing to the rapid growth of rhizomes, *C. riparia* keeps pace with the rising soil level with ease. The culms are numerous, 150 or more per square meter, and because of their rank growth tend to overshadow all other plants in the same area. Hence this species occupies space to the exclusion of everything else. Under these conditions the slower growing *C. stricta* is soon covered with soil and the invasion of *C. riparia* is quickly accomplished.

SPECIES WITH SHALLOW ROOT SYSTEMS.—Throughout the association are numerous species which produce roots at or near the surface of the soil. In relation to the tussock sedge they are entirely complementary. In ponds the runners of *Ranunculus delphinifolius* frequently cover the soil surface following recession of the water (fig. 1). In the more elevated portions of the association *Asclepias incarnata, Penthorum sedoides, Echinochloa crusgalli, Lycopus americanus*, and *Galium* sp. may occur. They seem to offer little competition to the tussock sedge.

LIST OF SPECIES.—The species found to occur in quadrats studied in the tussock association of the five counties previously mentioned are enumerated in the following list. The numbers preceding the species indicate their usual position in the association as follows·

(1) on the hydric side, (2) in the region of maximum development, and (3) on the mesic side or in transition areas. In general the nomenclature follows that of GRAY's manual (7); recent changes and segregations in certain genera, however, have been observed.

Species occurring in tussock association

Musci
(2) Barbula unguiculata (Huds.) Hedw.
(2) Brachythecium rivulare B. & S.
(1) Drepanocladus aduncus pseudofluitans Sanio
(3) Bryum sp.

Hepaticae
(1, 2) Marchantia polymorpha L.
(1, 2) Conocephalum conicum (L.) Dum.

Polypodiaceae
(3) Dryopteris thelypteris (L.) Gray

Equisetaceae
(2, 3) Equisetum arvense L.

Typhaceae
(1, 2) Typha latifolia L.

Sparganiaceae
(1) Sparganium eurycarpum Engelm.

Alismaceae
(1) Sagittaria latifolia Willd.
(1) Alisma plantago-aquatica L.

Gramineae
(3) Echinochloa crusgalli (L.) Beauv.
(3) Zizania aquatica L.
(3) Leersia oryzoides (L.) Sw.
(3) Phalaris arundinacea L.
(3) Muhlenbergia racemosa (Michx.) B.S.P.
(3) Phleum pratense L.
(3) Agrostis alba L.
(2, 3) Calamagrostis canadensis (Michx.) Beauv.
(3) C. neglecta (Ehrh.) Gaertn.
(3) Koeleria cristata Pers.

 (3) Spartina pectinata Link (S. michauxiana Hitchc.)
 (1) Phragmites communis Trin.
 (3) Poa pratensis L.
 (1) Glyceria nervata (Willd.) Trin.
 (2, 3) Bromus ciliatus L.
 (3) Hordeum jubatum L.
 (3) Elymus canadensis L.

Cyperaceae
 (3) Eleocharis obtusa (Willd.) Schultes
 (1, 2, 3) E. palustris (L.) R. & S.
 (3) E. acicularis (L.) R. & S.
 (1) Scirpus fluviatilis Gray
 (3) S. atrovirens Muhl.
 (3) S. lineatus Michx.
 (3) Carex bebbii Olney
 (3) C. sterilis Willd.
 (3) C. bromoides Schkuhr.
 (3) C. vulpinoidea Michx.
 (3) C. stipata Muhl.
 (3) C. sartwellii Dewey
 C. stricta Lam.
 (3) C. strictior Dewey
 (3) C. pennsylvanica Lam.
 (2, 3) C. lanuginosa Michx.
 (3) C. trichocarpa Muhl.
 (1, 2, 3) C. riparia W. Curtis
 (3) C. hystericina Muhl.
 (1) C. retrorsa Schw.
 (2, 3) C. rostrata Stokes

Araceae
 (2) Symplocarpus foetidus (L.) Nutt.

Lemnaceae
 (1) Lemna trisculca L.

Juncaceae
 (3) Juncus tenuis Willd.
 (3) J. dudleyi Wieg.

Liliaceae
    (2) Hemerocallis fulva L.
    (3) Lilium michiganense Farwell
    (2) Smilacina stellata (L.) Desf.
Iridaceae
    (1) Iris virginica L.
    (3) Sisyrinchium gramineum Curtis
Salicaceae
    (3) Salix nigra Marsh.
  (1, 2, 3) S. amygdaloides Anders.
    (1) S. lucida Muhl.
  (1, 2, 3) S. interior Rowlee (S. longifolia Muhl.)
  (1, 2, 3) S. cordata Muhl.
  (1, 2, 3) S. discolor Muhl.
  (1, 2, 3) S. petiolaris Smith
  (1, 2, 3) S. bebbiana Sarg. (S. rostrata Richards)
    (2) S. candida Flügge
Urticaceae
    (3) Urtica procera Muhl. (U. gracilis American authors)
    (3) Laportea canadensis (L.) Gaud.
    (2) Pilea pumila (L.) Gray
    (3) Boehmeria cylindrica (L.) Sw.
Polygonaceae
    (3) Rumex crispus L.
  (2, 3) R. verticillatus L.
    (1) Polygonum sp.
Ranunculaceae
    (1) Ranunculus delphinifolius Torr.
    (3) R. abortivus L.
    (2) R. pennsylvanicus L.f.
  (2, 3) Thalictrum dasycarpum Fich. & Lall.
    (3) Anemone virginiana L.
    (3) A. canadensis L.
  (1, 2) Caltha palustris L.
Cruciferae
    (3) Cardamine bulbosa (Schreb.) B.S.P.
    (3)·C. douglassii (Torr.) Britton

Crassulaceae
    (3) Penthorum sedoides L.
Saxifragaceae
    (3) Saxifraga pennsylvanica L.
    (3) Parnassia caroliniana Michx.
Rosaceae
    (2) Spiraea alba DuRoi
    (3) Potentilla monspeliensis L.
  (2, 3) Rosa carolina L.
Leguminosae
    (2) Trifolium procumbens L.
    (3) Lathyrus palustris L.
    (3) Apios tuberosa Moench.
Callitrichaceae
    (1) Callitriche palustris L.
Balsaminaceae
  (1, 2, 3) Impatiens biflora Walt.
Rhamnaceae
    (2) Rhamnus alnifolia L'Her.
Violaceae
  (2, 3) Viola cucullata Ait.
    (2) V. blanda Willd.
Onagraceae
    (2) Epilobium densum Raf.
Umbelliferae
    (1) Cicuta maculata L.
    (1) Sium suave Walt. (S. cicutaefolium Schrank.)
    (3) Zizia aurea (L.) Koch
    (2) Pastinaca sativa L.
    (3) Oxypolis rigidior (L.) Coult. & Rose
    (3) Angelica atropurpurea L.
Primulaceae
    (1) Lysimachia thyrsiflora L.
Gentianaceae
    (3) Gentiana andrewsii Griseb.
Asclepiadaceae
  (1, 2) Asclepias incarnata L.
    (3) A. syriaca L.

Verbenaceae
> (3) Verbena hastata L.

Labiatae
> (1) Scutellaria lateriflora L.
> (1) S. galericulata L.
> (3) Prunella vulgaris L.
> (2) Physostegia virginiana (L.) Benth.
> (3) Stachys tenuifolia var. aspera (Michx.) Fernald
> (2, 3) Lycopus virginicus L.
> (2, 3) L. americanus Muhl.
> (3) Mentha arvensis var. canadensis (L.) Briquet

Solanaceae
> (3) Solanum dulcamara L.

Scrophulariaceae
> (3) Linaria vulgaris Hill.
> (3) Scrophularia marilandica L.
> (2) Chelone glabra var. linifolia Coleman
> (3) Mimulus ringens L.

Plantaginaceae
> (3) Plantago major L.

Rubiaceae
> (2) Galium asprellum Michx.

Campanulaceae
> (2, 3) Campanula aparinoides Pursh

Lobeliaceae
> (2, 3) Lobelia syphilitica L.

Compositae
> (2, 3) Eupatorium purpureum L.
> (2, 3) E. perfoliatum L.
> (3) Solidago canadensis L.
> (3) S. serotina Ait.
> (2, 3) Aster novae-angliae L.
> (2, 3) A. puniceus L.
> (3) Ambrosia artemisiifolia L.
> (3) Xanthium sp.
> (3) Rudbeckia hirta L.
> (3) Helianthus grosseserratus Martens

(1) Bidens frondosa L.
(3) B. connata Muhl.
(3) Helenium autumnale L.
(3) Cirsium lanceolatum (L.) Hill

Succession

The tussock meadows in these areas constitute a stage in succession which is usually preceded by a reed-swamp stage and followed by the *Eleocharis-Juncus-Carex* complex of the typical hydrosere. The limits of the sedge meadow are usually well marked, owing to the growth form of its principal species, *Carex stricta*. The transition from open water (fig. 4) or from the reed-swamp stage to the tussock meadow is usually abrupt. Telescoping is occasionally apparent if the habitat conditions are changing rapidly or the topography is variable within the area. The tension zone along streams may be even more marked if the land rises gradually on both sides of the stream. In flat areas the association may be uniform throughout, although islands of relicts from preceding stages or of invaders indicating the stages to follow are occasionally present.

The transition to the following stage is usually a gradual one and is indicated more by the decreasing numbers of the tussocks than by the appearance of new or otherwise conspicuous species. The outer limits of the association are especially well marked in areas where rising ground is accompanied by a rapid increase in depth of the water table, in situations marked by abrupt changes in the soil type, and in grazed areas. Heavy pasturage may result in grassland directly bordering the tussock meadow. In all cases the boundary of the association may be determined by the presence or absence of the tussock sedge.

The *Eleocharis-Juncus-Carex* complex which usually succeeds the tussock association is dominantly herbaceous The most characteristic species are *Eleocharis palustris*, *Juncus dudleyi*, and *Carex bebbii*. The less frequent *Scirpus atrovirens* is usually present and is rendered conspicuous by its coarser growth and greater height. Intermingled with these species are various sedges and grasses: *Carex vulpinoidea*, *C. sartwellii*, *C. hystericina*, *Bromus ciliatus*, *Leersia*

oryzoides, Glyceria nervata, and *Calamagrostis canadensis.* A considerable array of dicotyledonous forms appears throughout the season. If the area is not subjected to heavy grazing, *Rumex crispus, Ranunculus abortivus, Penthorum sedoides, Saxifaga pennsylvanica, Lathyrus palustris, Mentha arvensis, Lycopus americanus, Mimulus ringens,* and *Lobelia syphilitica* are usually present. The autumnal aspect is usually characterized by a display of *Aster* and *Solidago* spp. which may completely obscure the meadow-like appearance of the complex.

In the tension zone between the tussock association and the *Eleocharis-Juncus-Carex* complex, *Carex stricta* usually shows a definite change in growth form. The pedestals become low mounds or merely tufts of culms growing at the soil level. Occasionally the spaces between the tufts become occupied by culms that have grown from rhizomes and a uniform distribution results. At this stage the tussock sedge is sometimes difficult to distinguish from the closely related *C. strictior,* which grows in mats rather than in tussocks. The deep green leaves of the former, however, serve to distinguish it from the paler glaucous foliage of the latter. By digging up the root system of *Carex stricta* in such places the remains of buried tussocks can sometimes be found.

In areas not subjected to grazing, succession may proceed through a shrub stage to a swamp forest. In such cases the most successful invaders of the tussock association are the willows and *Cornus stolonifera.* The shrubby willows, *Salix interior, S. petiolaris, S. discolor, S. lucida, S. bebbiana,* and *S. cordata,* are more aggressive than the tree willows, *S. amygdaloides* and *S. nigra.* All may be found in a single willow thicket bordering the tussock meadow or invading it from the pond side.

The willow stage is usually succeeded by a tree stage characterized by *Fraxinus nigra, F. americana, Quercus bicolor, Ulmus fulva,* and *Acer rubrum.* The shrubs *Alnus* sp., *Ribes grossularia, Cornus stolonifera, Sambucus canadensis,* and *Xanthoxylum americanum,* and the liana *Psedera quinquefolia* are frequent. The herbaceous layer may consist principally of sedges and grasses if the forest is open. In the shaded areas *Boehmeria cylindrica, Pilea pumila, Parietaria pennsylvanica, Laportea canadensis, Eupatorium purpureum,* and

Lycopus americanus are common. In open spaces in these forests tussocks sometimes persist.

In heavily grazed areas the *Eleocharis-Juncus-Carex* complex is generally absent. Bluegrass pasture directly borders the tussock association. The coarser culms of the sedge are apparently unpalatable to livestock, since the grass is eaten down to the bare earth before the sedges are touched. Occasionally the marginal tussocks around ponds are killed by livestock and remain as low, grass covered mounds. This has led to a generally accepted opinion among farmers that tussock meadows result from the trampling of cattle. The best development of the tussock meadow, however, appears in areas where domestic animals are never permitted, especially along railroad rights of way.

Environmental factors within the association

EVAPORATION

During the summer of 1929 atmometer studies were made in the area south of Cedarburg. Stations were established as follows: (1) in the flat tussock meadow, (2) in the hillside tussock meadow (on an east facing slope), (3) in the *Eleocharis-Juncus-Carex* complex, and (4) in the adjoining grassland. The atmometers were run in triplicate, with one bulb placed at 20 cm., one at 50 cm., and one at 120 cm. above the ground. In every case the third bulb was well above the level of the vegetation. The results are summarized in table III.

It is evident from the data obtained that evaporation above the vegetation varies little in rate over the entire area. The rates recorded are probably higher than the average, owing to the continued drought of that year. In a nearby beech-maple forest at a height of 20 cm. the evaporation rate varied from 6 to 14 cc. per day for the same period. The only effect of the drought on *Carex stricta* that was observed was the folding of the leaves on dry hot days. Long after the grassland had dried up and turned brown, the tussock meadow was still green and luxuriant.

The slight evaporation in the lower levels of the tussock meadow accounts for the persistence of many of the shade loving herbaceous

forms. Supplied with an abundance of moisture for their roots and subject to the greater humidity beneath the sedge cover, they were little affected by the drought. It was to be expected that evaporation near the ground would be influenced by the density and structure of the vegetation. In passing from station 1 to station 4 the increasing evaporation rate in the lower levels is well shown in table III. Correlated with this is the increasing openness of the stand and

TABLE III

AVERAGE DAILY EVAPORATION RATES (IN CC.) AT DIFFERENT LEVELS
IN TUSSOCK MEADOWS AND ADJACENT ASSOCIATIONS

STATIONS	HEIGHT OF ATMOMETER BULBS		
	20 CM.	˙50 CM.	120 CM.
(1) Flat tussock meadow...............	9.1	21.2	38.1
(2) Hillside tussock meadow............	11 3	24.6	39.0
(3) Eleocharis-Juncus-Carex complex.....	18.2	32.4	42.8
(4) Grassland........................	36.1	43.4	44.9

the decreasing evidence of stratification. The stratification that is evident in many tussock meadows is due in great part, as YAPP (15) has shown for other meadows, to variations in the rate of evaporation at different levels. The obvious conclusion is that evaporation plays little part in determining where a tussock meadow will grow, but that it must be considered a factor which helps determine the floristic composition of the meadow.

SOIL

The soil in these areas is usually a clay or silt loam. The surface layer of 12 to 15 inches is a black compact loam, rich in organic matter. Occasionally it is peaty in nature or shows a fibrous condition as a result of its plant remains. The color is brown or black, depending upon the degree of decomposition. The texture may vary according to the amount of material washed in from the surrounding uplands. The surface layer may be underlain by grayish or fine white sand, or occasionally by a zone of calcareous pebbles. The subsoil is a bluish silty clay which extends downward for several feet.

The surface of these areas is level or it slopes very gradually toward the adjoining drainage courses. Drainage is deficient, the surface water frequently standing for some time in the spring. Areas occurring along stream courses may be subject to overflow. Since the soil of the region is mainly derived from the Niagara limestone, the soils of the tussock meadows are alkaline, owing to the percolation of lime-containing water from the adjacent uplands.

SOIL REACTION.—More than 200 tests were made in the tussock meadows of Washington, Ozaukee, Milwaukee, and Waukesha counties. In every case the soil was found to be alkaline. The pH ranged from 7.2 to 8.1, with an average of 7.4. The lower soil horizons tended to be slightly more alkaline than the surface layer. The water of ponds and small streams showed approximately pH 8. No difference was noted between the soil reaction at the bases of tussocks and that in the tussocks themselves.

Although the soil of tussock meadows in the area studied was consistently alkaline, pH does not appear to be a limiting factor. Several tussocks were dug up and transplanted to the edge of Cedarburg Bog where the water is continuously acid. After two years the sedges were still growing, although subject to the competition of the bog vegetation, particularly *Phragmites communis*, *Carex filiformis*, and *Salix candida*. In the greenhouse *Carex stricta* grows luxuriantly in *Sphagnum* moss. When watered with tap water (from Lake Michigan) the moss showed a pH of 7.02. When watered with distilled water, or with water to which a slight amount of sulphuric acid had been added, the pH varied from 5.6 to 6.8. No differences in growth or color of the plants could be observed throughout this range.

SOIL MOISTURE.—The range of soil moisture in different stands of the tussock association was very great; in individual stands a marked seasonal variation was also observed. Owing to the large amount of organic matter in the spongy upper layer of the soil, the typically poor drainage, and the frequent occurrence of springs, an excess of moisture was present for a considerable part of the season. Based on the dry weight of the soil, the moisture content frequently exceeded 250 per cent. With the lowering of the water table the moisture content slowly dropped until it reached 60 to 80 per cent in late summer. In the subsoil the water content was much lower,

ranging from 20 to 60 per cent. In August, soil moisture in the *Eleocharis-Juncus-Carex* complex ranged from 40 to 60 per cent in the upper 6 inches and slightly less in the subsoil. Soil moisture was never reduced to the hygroscopic coefficient or to a point where it was unavailable for plant growth. Consequently it doubtless is never a direct limiting factor in the persistence of the tussock meadow.

WATER TABLE

A close correlation exists between the presence or absence of tussock meadows in an area and the level of the water table. In the areas of maximum development the water table stands at or near the surface throughout the season. The average depth of the water table in the low hummocky area south of Cedarburg, during the summer of 1929, was 4.8 inches. Rains frequently brought the water table to the surface but it always subsided within a few days. At no time during the entire summer did it fall below 11 inches.

The water table reaches its highest level in April and May as a result of the spring rains. At this time the water in ephemeral ponds may stand at a depth of 18 to 24 inches; in flat or gently sloping areas bordering stream courses it usually stands at the surface; on hillsides, where drainage is better, it stands a few inches below the surface. With the advance of summer it is gradually lowered until the minimum is reached in late August or early September, after which it again slowly rises.

Other workers have recognized the influence of the water level on the character of the vegetation. WARMING (10) states, "In many cases ground-water lies too high for certain plants; in other cases it is so far below that the roots cannot utilize it directly or indirectly; in still other cases it is at such a depth as to be reached by the roots at certain seasons, but not at others." STOUT (9) mentions, "It is universally recognized that the amount of water in a soil and the level at which the ground water stands are important direct factors in determining the character of the plant life which is present." It is possible to estimate with considerable accuracy the depth of the water table in these meadows by considering the season of the year and noting the character of the vegetation. "Islands" in the vegetation almost invariably indicate a difference in water level.

Evidence that the level of the water table has a direct influence on the character of the vegetation is furnished by drainage projects which are being carried out in some places. The placing of tile in a tussock meadow will result in the complete disappearance of the tussocks in a very few years. Measurements of the distance to the water table in such areas near Thiensville, Wisconsin, have shown that the water level is from 2 to 4 feet beneath the surface of the soil. Owners of the land thus drained state that 10 years ago the water stood at the surface for a greater part of the growing season and that well developed tussocks were present at that time.

Records of the water table in a small tussock area on a hillside south of Cedarburg were kept during the summers of 1931 and 1932. Small drainage ditches had been dug in order to make the area suitable for an apple orchard. The average level of the water table dropped approximately 6 inches a year. On September 20, 1933, the water table was 34 inches below the surface. Although the sedge vegetation was still present the area was characterized by a marked invasion of grasses, particularly *Poa pratensis*, and numerous weeds, including a few specimens of *Verbascum thapsus* and a great many *Solidago* spp. The dried stems of *Linaria canadensis* and *Prunella vulgaris* were also abundant. It is obvious that drainage is rapidly causing the disappearance of *Carex stricta* in this place.

Biotic factors

Grazing

The effect of grazing upon the tussock vegetation is noticeable only when the number of livestock present greatly exceeds the capacity of the pasture. Most of the better pasture lands are of the bluegrass type, tussock associations occasionally being present as local areas in the pasture. Pure tussock meadows are seldom used for grazing.

Most of the grazing is done by cattle and horses. Ordinarily they graze the tussocks only when the grass has been depleted. If the pasture is overstocked the complete disappearance of the tussocks may result. Around ponds the grass covered mounds, representing former tussocks, may be observed as a zone bordering those which

still remain. Where grazing is not intensive the tussocks may remain for a long time. Bluegrass invades the spaces between them and along with the grazing prevents their vegetative propagation by means of rhizomes. Under these conditions various weeds characteristically grow in the protection of the tussock crown.

Intensive grazing generally results in greater exposure of the soil and consequently in a decrease of moisture and a lowering of the

Fig. 8.—Winter aspect of tussocks with burned-over area at left; water table near surface in summer.

water table. Succession toward a bluegrass pasture is thus hastened. Succession toward the shrub and forest stages does not take place under the influence of grazing.

FIRE

Farmers commonly burn these areas each year after the vegetation has become dry (fig. 8). The opinion prevails that burning will cause the disappearance of the tussocks or improve the grazing qualities of the pasture. It does neither. Burning results in the removal of the dried leaves and culms which serve to fill in the spaces between the tussocks. The roots and rhizomes are not harmed to any great extent by fire. Burning, however, does permit an increased invasion of weeds into the area and drives out or kills the small game animals that frequent the meadow. It is a practice which might well be discontinued.

RODENTS

The destruction of tussocks is sometimes hastened by rabbits and mice. When food is scarce these animals sometimes eat the exposed roots and rhizomes just beneath the tuft of dead leaves. In the region of the cut ends of these organs a constriction in the pedestal occurs. The soil surrounding the roots remaining nearest the surface may crumble as a result of frost action or be washed away by rain. Renewed attacks of the rodents may so weaken the pedestal that it topples of its own weight. The destruction of tussocks by this means, however, is negligible.

Discussion

The conditions of the habitat occupied by the tussock sedge lie within the limits of tolerance of many other species; consequently the explanation of the dominance of *Carex stricta* in the tussock association must be sought primarily in the characteristics of the plant itself. Among the characteristics of a dominant WEAVER and CLEMENTS (12) include size, abundance, and duration. *Carex stricta* has all of these.

The chief competitors of the tussock sedge are species of similar form, the grasses and other sedges. Few of these are taller than *Carex stricta* and all lack the additional advantage in height contributed by a pedestal. In early spring when growth begins, the height of the pedestal represents an advantage which, under favorable conditions, is generally maintained. Thus species which grow concurrently with the tussock sedge are at a disadvantage from the beginning.

The abundance of culms is apparent even at a glance. Statistical studies show that *Carex stricta*, in areas of maximum development, may constitute almost 100 per cent of the vegetation. Abundant tillering takes place and new buds and culms may be produced throughout the summer.

The perennial nature of the tussocks and the considerable age to which they attain are factors which help to bring about their permanent occupancy of an area. The mass of dead leaves and the matted roots and rhizomes which are added to the soil each year tend to create a fibrous peat which is favorable to the continued growth of the sedge.

The cespitose habit contributes to the dominance of *Carex stricta* in still another way. It constitutes an admirable adaptation to a fluctuating water level. During that portion of the growing season when the water is well above the surface of the ground, the leaves and stems are seldom if ever submerged. Aeration of roots and rhizomes presents no problem, owing to their well developed aerenchyma. With the lowering of the water table and the consequent decrease in moisture supply in the region of the fibrous roots, the soil roots in the zone of saturation supply the necessary moisture. The demand for moisture during times of stress is lessened to some extent by the folding of the leaves.

FIG. 9.—Diagrammatic sketch of a *Carex stricta* tussock: *A*, leaves of current growing season; *B*, dead leaves of previous season; *C*, fibrous roots in earthy pedestal; *D*, rhizome; *E*, wirelike soil roots.

The factors which appear most to limit the growth of the tussock sedge are deficiency of water, soil type, shade, and grazing. A water depth greater than 18–24 inches in ponds is usually not conducive to the growth of tussocks. Likewise, *Carex stricta* is seldom present in areas where the water table falls more than 18–24 inches below the surface during the growing season. The rapid disappearance of

the association following drainage is evidence in support of this view. The moisture content of the soil may also be correlated with the average depth of the water table. In well developed tussock meadows the moisture content of the soil seldom falls below 60 per cent during the growing season. In adjacent associations where *Carex stricta* is absent it may fall as low as 15 per cent during periods of drought.

Soils low in organic matter never support tussock vegetation. In fact it appears that the soil must be somewhat peaty before the tussock sedge can even invade an area. Again, a water relation may exist, since peaty soils are capable of holding a high percentage of moisture. On the other hand the tussocks do not occur on sandy substrates, especially where the drainage is good and the moisture content falls rapidly with the advance of the dry season.

The effect of shade is especially apparent when the association is being invaded by shrubs and trees. Permanent quadrats, which were observed for a period of five years under medium sized trees of *Salix amygdaloides, S. nigra,* and *Ulmus americana* which had become established in an otherwise pure tussock association, show the disappearance of certain of the tussocks and a gradual invasion of herbaceous dicotyledonous forms, both in the spaces between the pedestals and in the crowns of the tussocks themselves. Quadrats in areas being invaded by shrubby willows show an even faster rate of disappearance.

Among the factors of the environment which do not appear to be limiting are evaporation and soil reaction. Although the evaporation rate varies in the various levels of the association and determines to an extent the nature of the secondary vegetation, it is little different at the upper level of the tussock meadow from the rate in adjacent associations. It does not appear to be a factor in succession. Similarly the hydrogen ion concentration of the soil in the different soil horizons and in the associations preceding and following the tussock association appears to remain fairly constant. Variations that occur are not large enough to be significant.

The omission of the tussock association in certain successions seems worthy of note. This is especially true of lakes with sandy shores and the margins of quaking bogs. In many of these areas the

proper soil is lacking although the water content may be high. Although *Carex stricta* has been grown in sand in the greenhouse, with difficulty, its ability to compete with sand-loving species in nature is doubtful. It is probable that this species has been excluded from the margins of many lakes as a result of its inability to migrate. Although the seeds are easily carried they have not been observed to germinate in nature, and all attempts to germinate them in the laboratory have failed. In addition, a surprisingly small number of fruiting plants have been found in the region studied.

The rapid succession that takes place around many lakes is also a factor which serves to exclude the slowly developing tussock sedge. In many instances the shrub stages are found at the very margins of lakes. In such cases light enters immediately as a limiting factor. Similarly the shrub stages in bogs may encroach rapidly upon the sedge vegetation, working destruction on species that depend more upon permanent establishment than upon migratory ability for their existence. The unstable substratum of the floating mat is also a poor foundation for a plant which needs firm anchorage for its pedestals. The effect of physiological conditions in bogs upon *Carex stricta* cannot be stated. Tussocks transplanted to bogs, however, have grown with a moderate degree of success for a period of two years.

Summary

1. The vegetation and environmental conditions of tussock meadows in southeastern Wisconsin were studied during the summers of 1929, 1932, and 1933.

2. Tussock meadows are developed along stream and pond margins, on springy hillsides, and in drained morainic depressions. They are found on soils showing a high percentage of organic matter.

3. Tussock meadows are dominated by a single species, *Carex stricta*, which produces tussocks that consist of a tuft of leaves and culms at the summit of a pedestal composed of roots, rhizomes, soil, and vegetable débris.

4. New tussocks are initiated by means of rhizomes which grow horizontally from the bases of old tussocks.

5. Individual tussocks may attain a height of 2 to 4 feet and a diameter of 8 to 30 inches. They may persist in an area for many years.

6. The leaves of *Carex stricta* show certain xeromorphic structures which indicate an adaptation to the excessive evaporation rates to which they are occasionally subjected. The roots and rhizomes show hydromorphic structures which indicate an adaptation to extremely moist conditions.

7. The tussock habit enables *Carex stricta* to persist in areas where the water level shows a marked seasonal fluctuation.

8. The tussock association is almost exclusively herbaceous. The most important secondary species are grasses and sedges. Dicotyledonous herbs may dominate in appearance but not in numbers at certain seasons.

9. *Carex stricta* furnishes marked competition for herbaceous species from the standpoint of both aerial and subterranean organs.

10. Progressive decrease in soil moisture and lowering of the water table lead to invasion by species of lower moisture requirements and the ultimate disappearance of the tussock association.

11. In succession, the tussock association is usually preceded by a reed-swamp stage and followed by a sedge-rush stage. The association may be invaded by willows which are eventually succeeded by a swamp forest.

12. Evaporation, annual fires, and soil reaction are not significant factors in succession.

13. Overgrazing may result in the disappearance of the tussock association and the appearance of grassland in its place.

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LITERATURE CITED

1. BRAUN-BLANQUET, J., and PAVILLARD, J., Vocabulaire de sociologie végétale. Montpellier. 1925.

2. DRUDE, O., Handbuch der Pflanzengeographie. pp. 292–294. Stuttgart. 1890.

3. HAYDEN, A., The ecologic subterranean anatomy of some plants of a prairie province in central Iowa. Amer. Jour. Bot. 6:87–105. 1919.

4. MACKENZIE, K. K., Notes on *Carex*. VIII. *Carex stricta* Lam. and its allies. Bull. Torr. Bot. Club 42:405–422. 1915.
5. MARTIN, LAWRENCE, The physical geography of Wisconsin (2nd ed.). pp. 226–231. Bull. 36. Ed. Series no. 4. Wisconsin Geol. and Nat. Hist. Surv. Madison. 1932.
6. NICHOLS, G. E., The vegetation of Connecticut. IV. Plant societies in lowlands. Bull. Torr. Bot. Club 42:169–217. 1915.
7. ROBINSON, B. L., and FERNALD, M. L., GRAY's New manual of botany. 7th ed. New York. 1908.
8. SHERFF, E. E., Vegetation of Skokie marsh. Bull. Illinois State Lab. Nat. Hist. 9:575–614. 1913.
9. STOUT, A. B., A biological and statistical analysis of the vegetation of a typical wild hay meadow. Trans. Wisconsin Acad. Sci., Arts and Letters 17:405–469. 1912.
10. WARMING, E., Oecology of plants. p. 45. Oxford. 1909.
11. WARMING, E., and GRAEBNER, P., Lehrbuch der ökologischen Pflanzengeographie. pp. 783–787. Berlin. 1930–1933.
12. WEAVER, J. E., and CLEMENTS, F. E., Plant ecology. p. 135. New York. 1929.
13. WEAVER, J. E., and FITZPATRICK, T. J., Ecology and relative importance of the dominants of tall-grass prairie. BOT. GAZ. 93:113–150. 1932.
14. WHITBECK, R. H., The geography and economic development of southeastern Wisconsin. p. 16. Bull. 58. Ed. series no. 6. Wisconsin Geol. and Nat. Hist. Surv. Madison. 1921.
15. YAPP, R. H., On stratification in the vegetation of a marsh, and its relations to evaporation and temperature. Ann. Bot. 12:275–319. 1909.

GROWTH STUDIES IN RELATION TO ULTRAVIOLET RADIATION[1]

B. N. SINGH, G. P. KAPOOR, AND R. S. CHOUDHRI

(WITH THIRTEEN FIGURES)

Introduction

The effect of ultraviolet radiation upon physiological processes has been investigated by animal physiologists and the treatment has found manifold applications in therapeutics, but our knowledge with respect to its influence upon plant processes is yet meager. The investigations so far reported appear to be more or less of a qualitative nature, dealing with either the indirect effect of these rays when cut off from sunlight or their direct influence when supplied in extra doses, notwithstanding the fact that the after-effects in the two cases could wholly be attributed to the same cause. Thus BONNIER and MANGIN (2), using appropriate filters, discovered the occurrence of photosynthesis under the influence of these rays, while URSPRUNG (10) failed to find even a trace of starch on irradiating leaves with a quartz mercury apparatus. SCHANZ (6) and POPP (5) have emphasized the detrimental effect of these rays upon plant growth, but TINCKER (9) stresses their utility in giving better stocky growth and higher yield. A lack of exact knowledge regarding the source of radiation, the intensity of the incident rays, the duration of exposures, and the precise stage in the ontogeny when such treatments will have maximum possibilities of growth and yield adds to the difficulty of assessing at its real value the efficiency of such rays in plant growth.

The present investigations were taken up during the years 1931–34. Pure strains of *Triticum vulgare* (var. Pusa 4), *Linum usitatissimum* (var. 1150 S), *Gossypium hirsutum* (var. New Amer. Upland), *Crotalaria juncea* (var. local), *Oryza sativa* (var. Jilhore), and *Nicotiana tabacum* (var. Pusa 177), raised in well drained, large sized

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containers under optimum conditions of nutrition, were subjected to ultraviolet rays of constant intensity from a 220-volt quartz-inclosed unscreened mercury vapor lamp held in a standard parabolic reflector, operated on 143 watts (130 volts, 1.1 amperes).

At successive intervals after treatment with these rays, the required number of plants from each of the series were selected in the morning hours and the fresh weight, leaf area, structural abnormalities, and other characteristics recorded. The material was finally incubated at 100°C. to yield the dry weight. A part of the material was prepared after LINK and TOTTINGHAM (3) for the estimation of acid-soluble carbohydrates by Pavy's method. Total nitrogen was determined by the modified Kjeldahl's method (4). In each case the after-effects of irradiation were studied in terms of morphological variabilities, growth activity, and the carbohydrate/nitrogen drifts at successive stages of the life-cycle.

Experimental findings

To study the effect of ultraviolet rays in their various aspects, a considerable number of experimental plants, of as nearly uniform size and weight as possible, were exposed to such rays for varying durations administered at shorter or longer intervals as follows:

A. AT INTERVALS SHORTER THAN A FORTNIGHT:

    (1) 5 minutes daily
    (2) 15 minutes on alternate days
    (3) 30 minutes on alternate days
    (4) 5 minutes weekly
    (5) 15 minutes weekly
    (6) 30 minutes weekly

B. AT INTERVALS LONGER THAN A FORTNIGHT:

    (1) 15 minutes fortnightly
    (2) 30 minutes fortnightly
    (3) 15 minutes twice in the life-cycle
    (4) 15 minutes once in the life-cycle

The experimental plants were exposed for the first time on the twenty-second day after germination when they were more or less

independent organisms, while subsequent irradiations depended to a large extent upon the particular dose the effect of which was under study.

A. Experiments on Irradiation of Plants at Intervals Shorter Than a Fortnight

Ten days after treatment, plants belonging to different sets begin to exhibit marked changes in their morphological features, the variations becoming more characteristic with the advance in age of the plants. Plants irradiated for 5, 15, and 30 minutes (except in case of *Linum* treated for 15 minutes on alternate days) fail to develop normally. Morphological differences in the general vigor, height, leaf area, stem development, and grain formation soon become apparent. The variations, although not very clearly defined in all the cases, when compared with the control, are more pronounced when either the time interval between two successive exposures is as short as only 24 hours or when the duration of each exposure is half an hour. Plants belonging to each of these sets are smaller in comparison with the control (table I) and have few or no branches. Leaves do not develop to the normal size and assume a pale yellowish green color within two to three weeks after the first treatment, showing a tendency to die prematurely. Attenuation is another outstanding feature in the unfavorable effects of these short-intervaled irradiations. When *Triticum* receives 5 minutes' daily irradiation, culm elongation is greatly inhibited resulting in the stunted growth of plants with reduced tillering, short narrow leaves, and small poorly developed heads (fig. 1). The variabilities are equally interesting in those sets where the duration of each exposure is as long as 30 minutes, even though the time interval is of a week's length. In addition to these variations in the general features, shedding of leaves and flowers is also common under this treatment. The resulting bolls in *Gossypium*, pods in *Crotalaria*, and ears in *Oryza* are ·'fewer in number and reduced in size, having few small shriveled seeds or none at all. In extreme cases only a naked rachis remains. The results obtained for *Crotalaria* are rather characteristic (fig. 2). Longer exposures are thus more damaging to the yield of plants than shorter ones supplied very frequently.

TABLE I

INFLUENCE OF ULTRAVIOLET RADIATION UPON PLANT GROWTH

ITEM	CONTROL	IRRADIATIONS AT SHORTER INTERVALS						IRRADIATIONS AT LONGER INTERVALS			
		DAILY	ALTERNATE DAYS		WEEKLY			FORTNIGHTLY		TWICE IN LIFE-CYCLE	ONCE IN LIFE-CYCLE
		5 MIN.	15 MIN.	30 MIN.	5 MIN.	15 MIN.	30 MIN.	15 MIN.	30 MIN.	15 MIN.	15 MIN.
Triticum vulgare											
Av. height per plant in cm	55.00±0.478	35.00±0.395	51.50±0.541	46.00±0.520		52.00±0.590		67.50±0.872	53.00±0.653		
Av. number of tillers per plant	3.40±0.121	2.10±0.140	2.70±0.016	2.30±0.035		3.00±0.142		4.60±0.170	3.20±0.050		
Final dry weight per plant in gm	8.07±0.174	3.93±0.082	6.12±0.142	5.10±0.105		7.75±0.165		8.49±0.178	8.84±0.134		
Av. length of heads in cm	8.50±0.101	6.07±0.080	6.50±0.091	6.00±0.072		7.80±0.041		10.30±0.135	8.30±0.220		
Av. yield of grain per plant in gm	3.35±0.065	1.56±0.024	1.36±0.046	2.16±0.051		2.49±0.035		3.76±0.072	3.16±0.055		
Absolute weight of 100 grains in gm	4.35±0.111	1.74±0.039	1.94±0.016	1.87±0.043		3.15±0.061		4.78±0.130	4.12±0.161		
Volume weight of grains per cc	0.66±0.071	0.21±0.005	0.28±0.008	0.22±0.012		0.54±0.041		0.68±0.085	0.61±0.054		
Period of blooming in days	50	36	43	43		50		50	50		
Duration of floral development in days	21	14	14	14		14		14	14		
Fruiting stage in days	71	50	57	57		64		64	64		
Harvesting period in days	106	78	92	85		99		106	99		
Linum usitatissimum											
Av. height per plant in cm	32.50±0.540	25.00±0.327	34.00±0.463	39.50±0.395		30.50±0.452		46.00±0.475	50.00±0.680		
Final dry weight per plant in gm	2.93±0.055	1.40±0.023	3.64±0.048	2.33±0.042		2.33±0.042		6.57±0.160	7.64±0.189		
Av. yield of grain per plant in gm	1.94±0.032	0.30±0.008	2.02±0.051	1.08±0.020		1.10±0.015		3.60±0.062	3.95±0.081		
Period of blooming in days	50	36	36	36		42		50	50		
Duration of floral development in days	28	14	14	14		14		14	14		
Fruiting stage in days	78	50	50	50		56		64	64		
Harvesting period in days	106	78	92	85		99		106	99		

TABLE I—*Continued*

ITEM	CONTROL	DAILY 5 MIN.	ALTERNATE DAYS 15 MIN.	ALTERNATE DAYS 30 MIN.	WEEKLY 5 MIN.	WEEKLY 15 MIN.	WEEKLY 30 MIN.	FORTNIGHTLY 15 MIN.	FORTNIGHTLY 30 MIN.	TWICE IN LIFE-CYCLE 15 MIN.	ONCE IN LIFE-CYCLE 15 MIN.
Gossypium hirsutum											
Av. height per plant in cm	77.50 ± 0.840				72.50 ± 0.780	56.50 ± 0.620	45.00 ± 0.572				
Final dry weight per plant in gm	105.00 ± 0.820				87.60 ± 0.542	51.90 ± 0.560	30.60 ± 0.401				
Av. number of bolls per plant	10.50 ± 0.210				8.20 ± 0.151	7.00 ± 0.120	5.10 ± 0.052				
Av. weight of seed-cotton per plant in gm	15.50 ± 0.248				11.52 ± 0.127	7.12 ± 0.102	4.30 ± 0.082				
Av. weight of cotton-seed per plant in gm	10.40 ± 0.230				8.06 ± 0.105	4.56 ± 0.087	3.40 ± 0.040				
Period of blossoming in days	71				66	66	64				
Duration of floral development in days	28				26	26	26				
Fruiting stage in days	99				92	92	90				
Harvesting period in days	127				120	120	120				
Crotalaria juncea											
Av. height per plant in cm	106.20 ± 0.780				87.50 ± 0.560	75.00 ± 0.450	68.70 ± 0.405			110.50 ± 0.630	108.00 ± 0.845
Final dry weight per plant in gm	6.20 ± 0.155				4.44 ± 0.150	4.00 ± 0.105	2.16 ± 0.032			9.53 ± 0.225	8.35 ± 0.090
Av. number of pods per plant	10.80 ± 0.065				3.30 ± 0.170	2.80 ± 0.135	2.00 ± 0.083			15.70 ± 0.213	11.92 ± 0.110
Av. yield of grain per plant in gm	1.84 ± 0.015				1.33 ± 0.013	1.01 ± 0.021	0.61 ± 0.004			1.98 ± 0.035	1.921 ± 0.030
Period of blossoming in days	43				42	40	40			43	43
Duration of floral development in days	21				17	17	17			14	14
Fruiting stage in days	64				59	57	57			57	57
Harvesting period in days	99				93	92	92			98	98
Oryza sativa											
Av. height per plant in cm	37.50 ± 0.556				30.00 ± 0.482	25.50 ± 0.491	22.50 ± 0.350				
Final dry weight per plant in gm	3.91 ± 0.072				2.23 ± 0.081	1.60 ± 0.045	0.52 ± 0.013				
Av. yield of grain per plant in gm	0.97 ± 0.031				0.80 ± 0.030	0.51 ± 0.013	0.20 ± 0.005				
Period of blossoming in days	71				66	64	64				
Duration of floral development in days	16				14	14	14				
Fruiting stage in days	87				80	78	78				
Harvesting period in days	106				101	99	99				

Figs. 1, 2.—Fig. 1 (above), fully developed plants of *Triticum:* (1) control, (2) 5 minutes every day, and (3) 15 minutes fortnightly. Fig. 2 (below), fully developed plants of *Crotalaria:* (1) control, (2) 5 minutes weekly, (3) 30 minutes weekly.

There is a tendency for these short-intervaled irradiations to inhibit vegetative growth and force early flowering and maturity, associated with reduced yield of grain (table I). Treatments administered more frequently (daily) or given for longer durations (30 minutes per exposure) induce such changes to a greater extent. Average weights of the oven-dried entire plants taken at successive stages further indicate that, with the exception of *Linum*, all other plants thus treated show a lack of dry matter accumulation at all stages following treatment (fig. 3, table I). *Linum* exhibits an increase in the dry weight for several successive weeks during the course of treatment (fig. 4) and is thus more resistant to such doses.

B. EXPERIMENTS ON IRRADIATIONS OF PLANTS AT INTERVALS LONGER THAN A FORTNIGHT

When the time interval between two successive exposures is increased to a fortnight or more, even 30 minutes' dose induces rapid development and increased growth in *Linum*. In *Triticum* 15 minutes' dose supplied at similar intervals induces a highly vegetative growth with increased tillering and large greenish leaves in contrast to control plants (fig. 1). Such irradiations also produce longer heads, apparently as a result of abnormal elongation of the internodes of the rachis. The average weight of grains per plant after such irradiations exceeds that of the control plants. The grains produced are also superior in quality, in so far as their absolute and volume weights are concerned. The plants are unusually tall (table I).

Of the plants thus studied, *Crotalaria* is found to be exceedingly sensitive to the action of these rays, for when irradiated even once in the age-cycle, appreciable increments in the size of both the foliage and the floral organs are noticed. Increased height with high grain yield is observed in those plants which are exposed twice in the lifecycle, once as usual on the twenty-second day after germination and subsequently after a fortnight; that is, a few days preceding flowering (table I). This indicates that a single irradiation in *Crotalaria* is by no means sufficient to ensure maximum possibilities of growth and yield. *Nicotiana* is almost equally sensitive to such rays. In most of the plants thus tested the periods of flowering and maturity

are not markedly affected, while the dry matter accumulation and yield are considerably increased (tables I, II). Doses of 30 minutes at intervals of a fortnight are slightly harmful to the growth of *Triticum.*
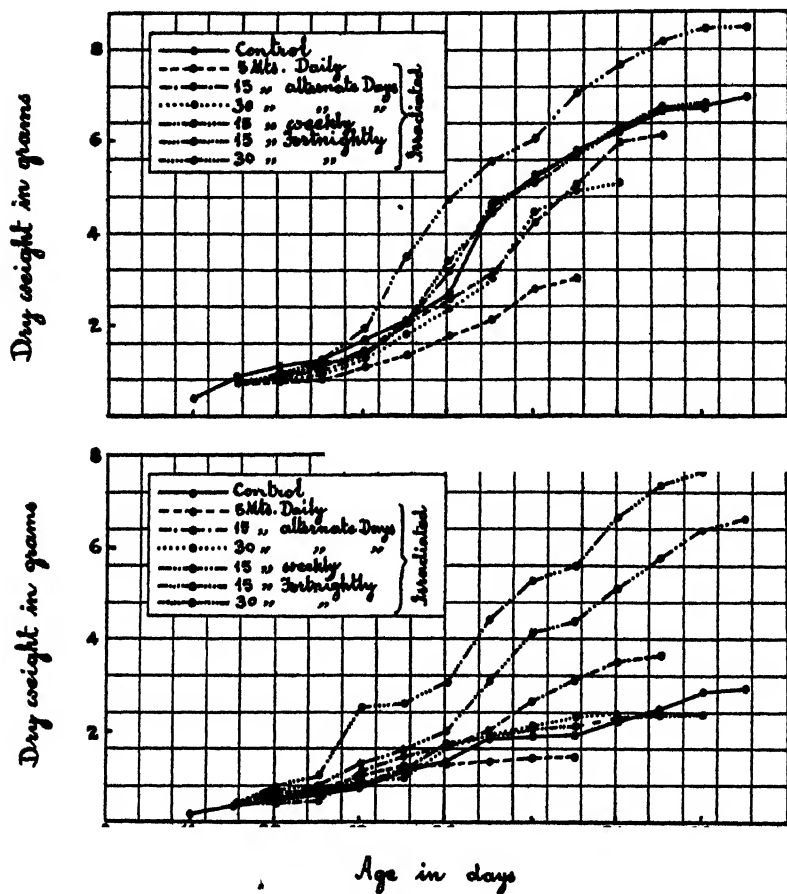
Figs. 3, 4.—Fig. 3 (above), time-weight curves for *Triticum* showing relative increment in dry matter accumulation of entire plant after irradiation. Fig. 4 (below), time-weight curves for *Linum* showing relative increment in dry matter accumulation of entire plant after irradiation.

SEED TREATMENT.—Seeds of *Nicotiana* after soaking in 1 per cent sodium chloride for an hour were dried at laboratory temperature and exposed for 5, 10, and 20 minutes respectively to ultraviolet rays.

Seeds treated for 5 and 10 minutes are found to germinate and grow more vigorously and in larger numbers than either the control or those treated for 20 minutes, which in their turn exhibit poorer germination even than the untreated ones (table II). The subsequent growth and other morphological characteristics also undergo marked changes in response to variations introduced during the
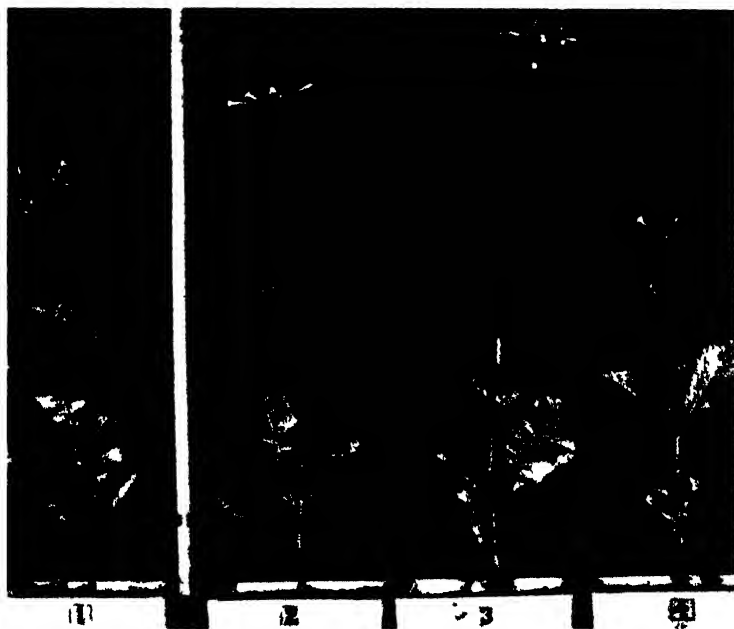
Fig. 5.—Fully developed plants of seed-treated *Nicotiana:* (1) control, (2) 5 minutes, (3) 10 minutes, (4) 20 minutes.

early seedling stage in the various experimental sets. Seeds treated for 5 and 10 minutes respectively present taller plants greatly surpassing the controls (fig. 5), with bigger leaves and a more extensively developed root system. Of these, a dose of 5 minutes' duration has only a transitory effect, giving increased leaf yield (table II). Exposure of 10 minutes' duration, on the other hand, accelerates equally the development of foliage and inflorescence, bringing considerable increase in both the leaf and the grain yield. Blossoming and maturity are not much affected by such irradiations. Plants

belonging to 20 minutes' seed-treated sets on the other hand are forced into reproductive stage so rapidly that there is an insufficient development of leaves and stems (fig. 5). They matured one week earlier than the control plants and gave poor yield of both leaf and grain (table II).

ABSOLUTE GROWTH AND GROWTH RATE.—Control time-weight curves for the crop plants under investigation are more or less of a sigmoid type (figs. 3, 4), a detailed description of which has already appeared (7). Comparing these curves with those obtained for irradiated plants, a striking similarity in their forms is noticed which indicates that the dynamics of growth is not fundamentally affected in response to ultraviolet radiation. The consistent variation in their level under identical conditions of environment, however, points to the change being conditioned by a variation in the internal factors of the plant as a result of irradiation, leading to a greater accumulation of dry matter in some cases and lesser in others.

The relative growth rate curves belonging to the experimental series of plants depict a greater slope than their respective checks in the week following the treatment, indicating that the immediate after-effect of these rays is to retard growth (figs. 6–9). The maximum growth point in each case is attained on the approach of flowering, and hence irradiations at intervals shorter than a fortnight (except in *Linum*) are associated with earlier growth maxima in contrast to check plants. A stricter comparison of these curves reaffirms that the series of plants producing higher yield are essentially those preceded by higher growth maxima and vice versa.

CARBOHYDRATE/NITROGEN DRIFTS.—To obtain some indication regarding the developmental variations, and also to reveal the physiological balance in plants on exposure to ultraviolet rays, carbohydrate/nitrogen determinations were made. Figure 10 shows that the percentage of carbohydrates at similar stages in the control and seed-treated series of *Nicotiana* varies from set to set, although the nature of the curves remains identical inasmuch as they are all characterized by a preflowering maximum. Five and 10 minutes' seed-treated plants are associated with higher carbohydrate percentages than the check, while those whose seeds received treatment for 20 minutes show lower percentages. As far as the nitrogen is concerned,

TABLE II

RELATIVE EFFECTS OF SEED AND PLANT IRRADIATION IN NICOTIANA TABACUM

ITEM	CONTROL	SEED TREATMENT			PLANT TREATMENT ONCE IN LIFE-CYCLE
		5 MINUTES	10 MINUTES	20 MINUTES	
Percentage capacity for germination	70	79	91	46	....
Speed of germination in days	20	18	15	25	....
Average height per plant in cm	68.75 ±0.491	81.25 ±0.640	87.50 ±0.620	62.50 ±0.385	84.50 ±0.530
Final dry weight per plant in gm	26.92 ±0.206	31.52 ±0.357	39.10 ±0.239	25.70 ±0.255	36.28 ±0.242
Average leaf yield per plant in gm	6.45 ±0.110	8.10 ±0.184	11.50 ±0.138	5.20 ±0.120	10.90 ±0.143
Average grain yield per plant in gm	0.67 ±0.012	0.62 ±0.013	1.88 ±0.020	0.66 ±0.010	1.45 ±0.045
Absolute weight of 100 grains in gm	0.0035 ±0.0001	0.004 ±0.0005	0.005 ±0.0002	0.003 ±0.0005	0.004 ±0.006
Volume weight of yield grains per cc	0.367 ±0.003	0.368 ±0.007	0.423 ±0.011	0.352 ±0.015	0.400 ±0.042
Period of blossoming in days	94	94	94	89	94
Duration of floral development in days	14	12	12	12	12
Fruiting stage in days	108	106	104	101	106
Harvesting period in days	129	127	127	122	127

there is a progressive decrease in its percentage with increase in the duration of exposures (fig. 11). Unlike carbohydrates, therefore, in none of the irradiated sets does nitrogen percentage increase beyond
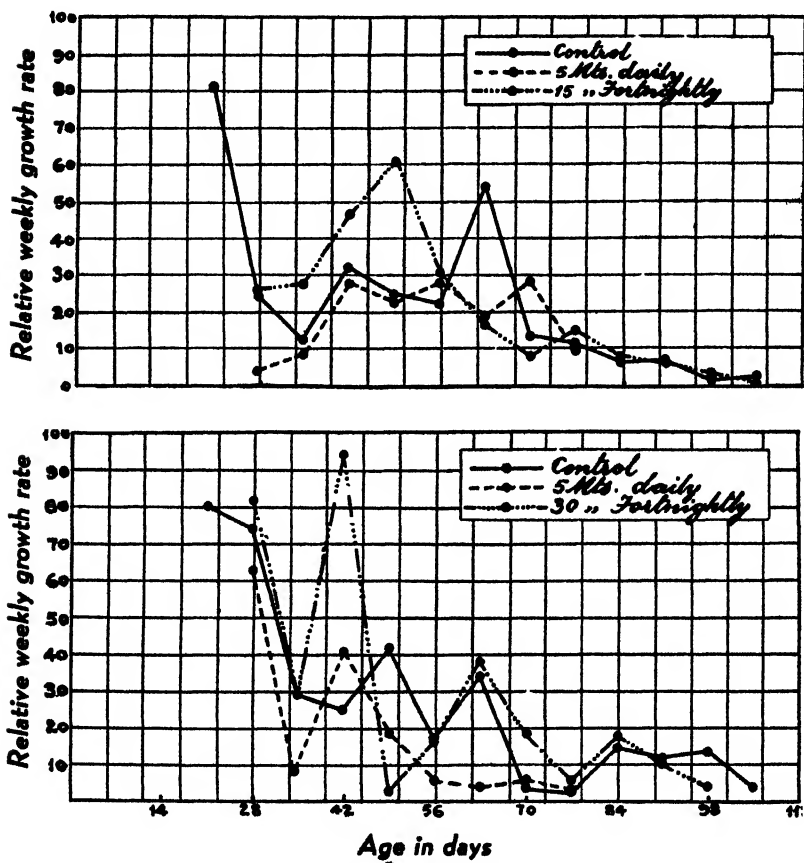
Figs. 6, 7.—Fig. 6 (above), relative growth rate curves of control, 5 minutes daily, and 15 minutes fortnightly treated plants of *Triticum*. Fig. 7 (below), relative growth rate curves of control, 5 minutes daily, and 30 minutes fortnightly treated plants of *Linum*.

that of the check. The carbohydrate/nitrogen ratio curves (fig. 12), however, resemble those for carbohydrates belonging to similar series. Plants of 10 minutes' seed-treated series giving high grain yield are characterized by the highest carbohydrate/nitrogen ratio,

while those of the 20 minutes' series where the development of plants is inadequate are associated with very low ratio.
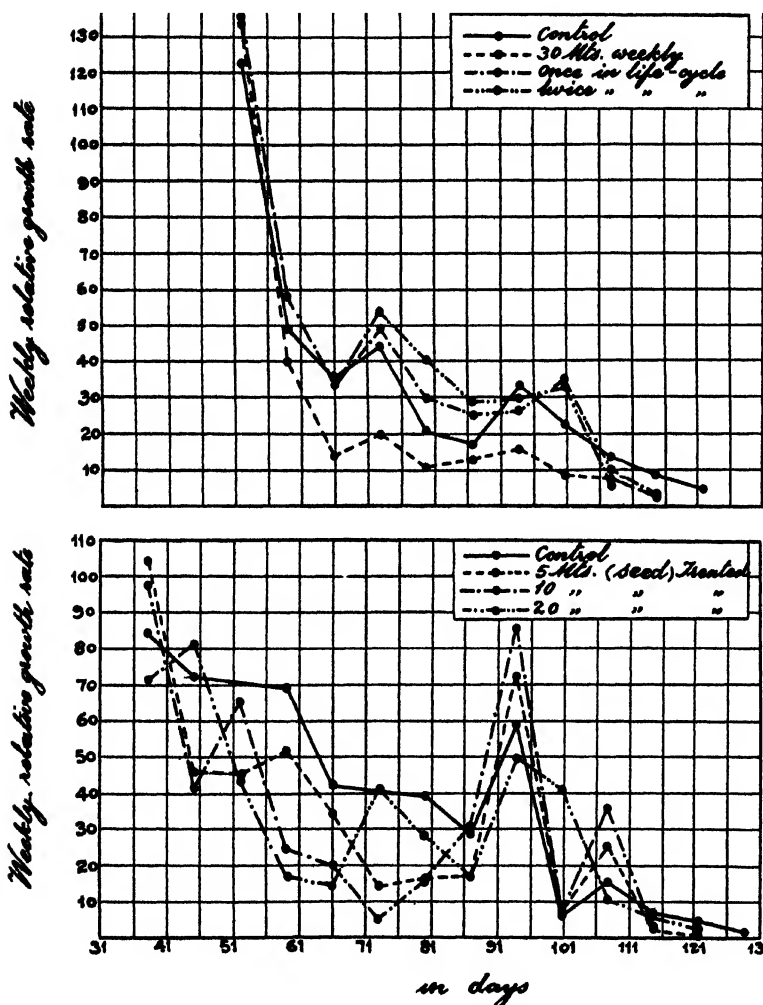
Figs. 8, 9.—Fig. 8 (above), relative growth rate curves of control, 30 minutes weekly, once in life-cycle, and twice in life-cycle treatments in *Crotalaria*. Fig. 9 (below), relative growth rate curves of seed-treated *Nicotiana*.

NET ASSIMILATION RATE.—The values of net assimilation rate calculated from dry weight and leaf area data are taken into account to

explain the induced variations brought about by ultraviolet rays in the carbohydrate and nitrogen accumulations. Figure 13 indicates that the assimilation rates also undergo marked changes like those obtained in the carbohydrates. Five and 10 minutes' seed-treated
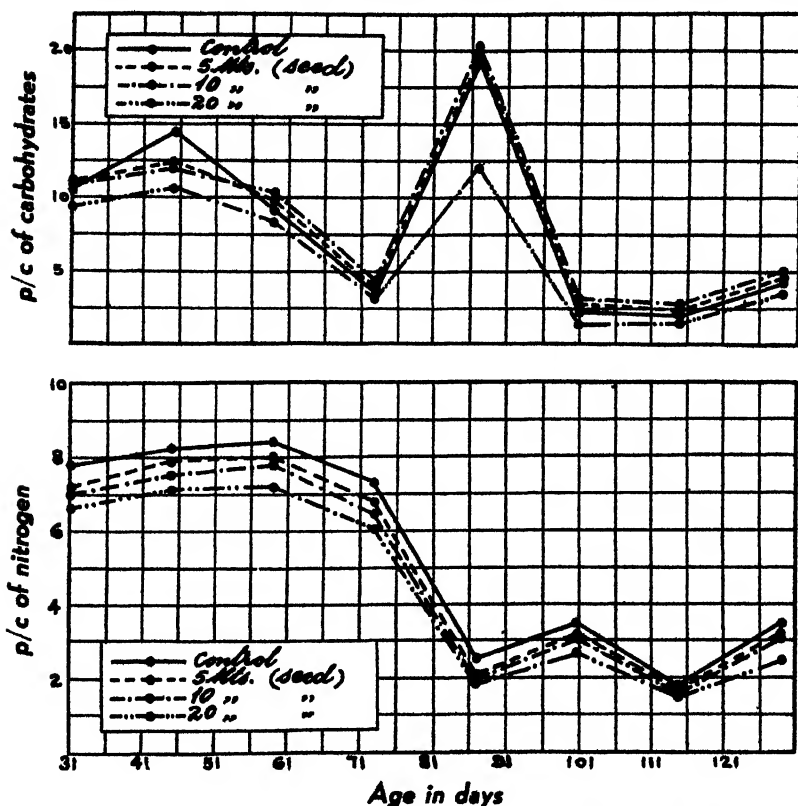
Figs. 10, 11.—Fig. 10 (above), carbohydrate percentages in seed-treated plants of *Nicotiana*. Fig. 11 (below), nitrogen percentages in seed-treated plants of *Nicotiana*.

plants, therefore, depict higher while those developing from seeds treated for 20 minutes depict lower rates, in comparison with the control. The relative growth rate curves for the seed-treated and control series show more or less similar features, inasmuch as the maxima in the two cases fall at the same stage (figs. 9, 13). High assimilation rates are therefore correlated with high carbohydrate accumulation and high growth rates, and vice versa.

Results obtained in the present investigation thus show that variations in the growth and yield of plants on exposure to ultraviolet radiation are associated with fluctuations in the assimilation rate and consequent changes in the nutrient level of the plants.
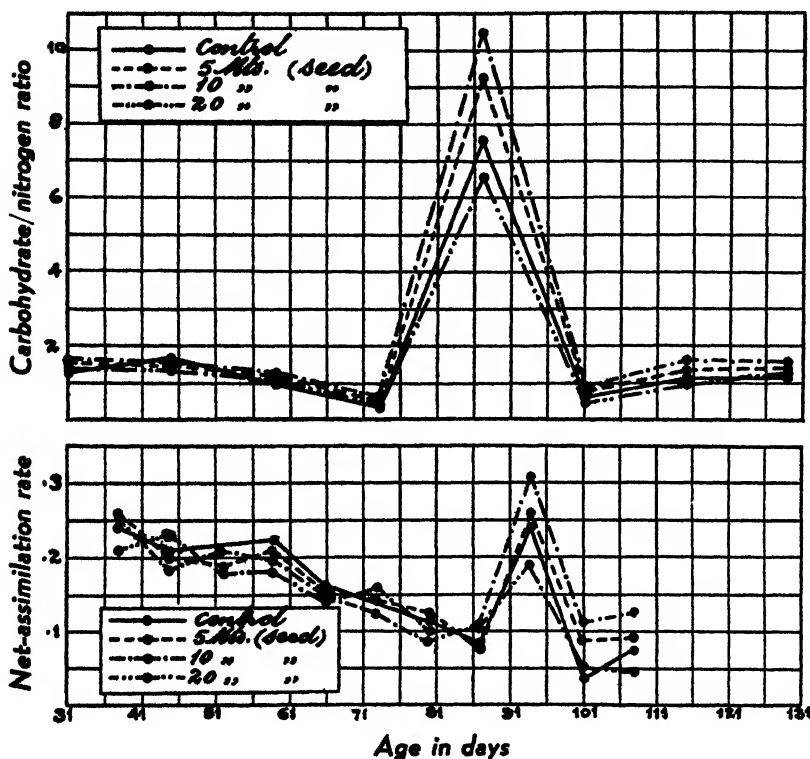
Figs. 12, 13.—Fig. 12 (above), carbohydrate/nitrogen ratio in seed-treated *Nicotiana*. Fig. 13 (below), net assimilation rate of seed-treated plants of *Nicotiana*.

In the light of these experimental findings it is to be admitted that certain doses of ultraviolet rays are beneficial in increasing the growth and yield of plants. BENEDICT (1) has recently shown that ultraviolet rays of 2900–3100 Å are useful to the growth of plants while still shorter waves produce harmful effects. But since such rays are rarely transmitted through the epidermis, as shown by BENEDICT, the extent of resulting harm due to these rays may be considered to be markedly less. In the present case, however, the

range of wave length is decidedly greater since no filters were used, but the plants raised under natural conditions of environment are subjected to such rays only occasionally, and hence in some cases increase in growth and yield takes place. The differential behavior in the growth response of different crop plants is explained on their specific ultraviolet requirement. Like x-radiation (8), therefore, ultraviolet rays also are useful when administered in suitable doses in initiating profitable crop production.

Summary and conclusions

1. The present investigations aimed at a quantitative analysis of the effect of ultraviolet radiation, administered in varying doses, frequently or occasionally, upon the growth and yield of certain crop plants. The definable after-effects consequential to such treatments are estimated in terms of morphological changes, growth behavior, the drifts in the carbohydrate and nitrogen percentages, and the net assimilation rate at successive stages of growth during the entire life-cycle of the crops.

2. Exposure of plants to ultraviolet rays for a period of 5 minutes or more at intervals shorter than a fortnight is detrimental to their growth and yield. The more frequent the exposures or the longer the duration of each exposure, the greater is the harm incurred. Longer exposures at one time are more damaging to the yield of some plants than shorter ones supplied frequently. Occasional treatments of 15 minutes' duration are productive of good growth.

3. Seed treatment for 10 minutes or less accelerates germination and induces luxuriant growth in plants while longer treatments of 20 minutes' duration retard both germination and development.

4. In all cases where early flowering and maturity are the result of ultraviolet exposures, the plants are characterized by stunted growth and reduced yield; whereas under treatments giving better growth and yield as compared with the control, the normal period of vegetative and reproductive growth remains more or less unchanged.

5. The yield of plants subjected to ultraviolet rays is proportional to the magnitude of the time-weight curves as well as to the maximum height of the growth rate curves.

6. Variations in the growth and yield of crops on exposure to ultraviolet rays are explained on the basis of fluctuations in the net assimilation rate and the carbohydrate/nitrogen ratio. The response to these rays appears to be different in different plants.

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LITERATURE CITED

1. BENEDICT, H. M., Effect of ultraviolet radiation on growth and on the calcium and phosphorus contents of plants. BOT. GAZ. 96:330–341. 1934

2. BONNIER, G., and MANGIN, L., L'action chlorophyllienne dans l'obscurité ultra-violette. Compt. Rend. Acad. Sci. Paris 102:123. 1886.

3. LINK, K. P., and TOTTINGHAM, W. E., Effect of the methods of desiccation on the carbohydrates of plant tissues. Jour. Amer. Chem. Soc. 45:439–446. 1923.

4. Methods of Analysis of the Association of Official Agricultural Chemists. 3d ed. 1930.

5. POPP, H. W., A physiological study of the effect of light of various ranges of wavelength on the growth of plants. Amer. Jour. Bot. 13:706–737. 1926.

6. SCHANZ, F., Wirkungen des Lichts verschiedener Wellenlänge auf die Pflanzen. Ber. Deutsch. Bot. Ges. 37:430–442. 1919.

7. SINGH, B. N., The basis of aging, rejuvenation and death. Being an experimental study into the physiology and chemistry of plant material. Malviya Commemoration volume, Indian Press. Allahabad, India. 1932.

8. SINGH, B. N., and CHOUDHRI, R. S., Induced morphological, physiological and chemical variations following seed exposure to x-radiation in *Nicotiana tabacum*. Proc. Ind. Acad. Sci. Vol. I. no. 8. 1935.

9. TINCKER, M. A. H., Some experiments with ultra-violet ray glasses. Jour. Roy. Hort. Soc. 55:79–87. 1930.

10. URSPRUNG, A., Über die Stärkebildung im Spektrum. Ber. Deutsch. Bot Ges. 35:44–69. 1917.

EFFECTS OF BARIUM SALTS UPON ASPERGILLUS NIGER AND THEIR BEARING UPON THE SULPHUR AND ZINC METABOLISM OF THE FUNGUS IN AN OPTIMUM SOLUTION

ROBERT A. STEINBERG

During the course of investigations on the nutrition of *Aspergillus niger* the observation was made that, although as found by JAVILLIER (2), the addition of small quantities of barium salt to the nutrient solution was without effect upon the growth of the fungus, larger amounts resulted in cultures identical in most respects with the minus iron and minus zinc cultures (4). Since the results obtained bore directly upon the interpretation of the influence of iron and zinc on the development of the fungus, additional information was sought to clarify their relation to the heavy metal and sulphur nutrition of the organism.

The nutrient solution employed in these investigations contained 1000 cc. water redistilled through pyrex glass, 50 gm. of sucrose, 2 gm. of ammonium nitrate, 0.48 gm. of dipotassium phosphate, and 0.62 gm. of magnesium nitrate ($Mg(NO_3)_2 . 6H_2O$). To this solution also were added 0.15, 0.12, 0.03, and 0.02 mg. per liter of iron, zinc, copper, and manganese as sulphate, respectively. The solution contained about 1 mg. of sulphur per liter. Its pH was 5.64. A solution having a pH of 4.16 was employed for the barium precipitation experiments. It was obtained by substitution of 0.75 gm. of KH_2PO_4 for the K_2HPO_4 of the preceding formula.

Table I indicates the effects of the use of sulphur in various forms upon the growth and development of the fungus. The data are average values for duplicate cultures in 200 cc. pyrex Erlenmeyer flasks containing 50 cc. of nutrient and grown at 34.7°–35.0° C. for 6 days.

Increases in growth followed upon addition to the nutrient solution of sulphur as sulphuric acid, sodium sulphide, and sodium sulphate. The last appeared most effective in supplying the needs

of the organism for sulphur. The maximum yield occurred with 20 mg. of sulphur per liter. Analogous values for sulphur supplied as sulphuric acid are almost identical at the lower sulphur concentrations. The first indication of a divergence is at 20 mg. of sulphur per liter. At this point the yields with sulphur as sulphuric acid begin to diminish, presumably as the effect of an excessive increase in acidity.

The pH values at harvest showed a relation similar to those of the yields, whereas the results with sporulation were quite parallel

TABLE I

EFFECT OF ADDITION TO THE NUTRIENT SOLUTION OF SULPHUR
UPON GROWTH AND DEVELOPMENT OF A. NIGER

SULPHUR MG./L	SULPHURIC ACID			SODIUM SULPHIDE			SODIUM SULPHATE		
	YIELD (MG.)	pH	SPORES	YIELD (MG.)	pH	SPORES	YIELD (MG.)	pH	SPORES
....	19.3	3.42	1,bl*	18.0	3.14	1,j	19 9	3.12	1,j
5	344.3	2.00	5,bl	......	......	......	353.3	2.00	5,bl
10	616.8	1.80	3,bl	42.2	2.97	2,j	593.1	1.77	5,bl
15	820 2	1.78	3,bl	......	......	......	787.0	1.78	4,bl
20	801.8	1.92	3,bl	63.0	2.75	3,j	881.5	1.82	3,bl
25	781.8	2.03	3,bl	......	......	......	865.6	1.88	3,bl
30	758.8	2.21	3,bl	93.1	2.54	4,j	868.3	1.94	3,bl
35	732.8	2.24	3,bl	......	......	......	864.1	1.88	3,bl
40	703.8	2.31	3,bl	127.8	2.43	5,j	870.4	1.94	3,bl
45	......			......			884.1	1.92	3,bl

* Sporulation is indicated as numbers o (sterile) to 5 (entirely covered with spores); their color by the initial letter of the shades white, yellow, tan, brown, black, and jet.

for the two series. The ratios of maximum to minimum yield were 44.3 for sodium sulphate and 42.5 for sulphuric acid. These values correspond to a decrease in yield upon non-addition of sulphur of 97.7 and 97.9 per cent from the maximum with sulphur, respectively.

Despite the increases in yield with sodium sulphide, there is no evidence that sulphur in the form of sulphide may participate in the sulphur nutrition of the fungus, although the sulphide ion evidently is innocuous in the concentrations employed. The readiness with which sulphides undergo oxidation renders it probable that traces of sulphate were present in the salt, or were formed during

the course of the experiment. This interpretation is in agreement apparently with the data given in table II.

These statements should not be assumed to imply that only sulphur as sulphate ion is available to the organism. PFEFFER points out in his textbook that not only sulphates but also "isothionic" acid, taurine, and certain sulpho-acids may serve as sources of sulphur even with the higher plants. The data in table II indicate

TABLE II

EFFECT OF BARIUM SALT UPON UTILIZATION OF SULPHUR
IN VARIOUS FORMS BY A. NIGER

BACL₂ 2H₂O GM./L	40 MG. S/L (H₂SO₄)			30 MG. S/L (NA₂S)			1 MG. S/L (IMPURITY)			40 MG. S/L (CYSTINE)		
	YIELD (MG.)	pH AT HAR-VEST	SPORES	YIELD (MG.)	pH AT HAR-VEST	SPORES	YIELD (MG.)	pH AT HAR-VEST	SPORES	YIELD (MG.)	pH AT HAR-VEST	SPORES
....	703 8	2 31	4,bl	93.1	2 54	5,j	19 3	3 42	1,j	889 7	2 15	4,bl
0.1	751 8	2 05	4,bl	80 0	2 73	4,j	20 1	3.18	1,j	829.3	1 92	4,bl
0 2	727.9	1.84	4,bl	59.7	2 93	4,j	19.5	3 18	1,j	814 9	......	4,bl
0.3	630 1	1 84	4,bl	51 5	2 95	4,j	18.5	3.18	1,j	842 6	1.88	4,bl
0 4	353 4	1 88	5,bl	57.6	3 03	4,j	......	......	......	......	......	......
0.5	317.0	1 98	5,bl	......	......	......	......	......	......	......	......	......

the suitability of cystine as a sulphur supply. Other data on hand have shown sodium thiosulphate to be an excellent source of sulphur for the fungus, while even potassium thiocyanate may be utilized, although with difficulty.

Non-addition of sulphur, moreover, results in the formation of submerged and slimy translucent hyphae that seem unable to form a felt upon the surface of the nutrient solution. Spore formation first increases and then decreases almost to the point of complete suppression with progressive decreases in sulphur concentration.

The effects of the addition of barium salt to the nutrient solution containing sulphur in various forms are shown in table II. Addition of barium salt to the solution containing sulphur as sulphate ion resulted in the formation of a white precipitate which apparently was BaSO₄. Small quantities of barium salt brought about slight increases in yield and acidity at harvest, since sulphuric acid was present at more than double the optimum concentration. Larger quantities, however, brought about a progressive decrease in yield and acidity at harvest accompanied by increased sporulation. The

macroscopic appearance of the felts with increasing concentrations of barium salt became more and more similar to those of the minus sulphur, minus iron, and minus zinc cultures. At barium concentrations sufficient for precipitation of all sulphate ion, the barium cultures, the minus sulphur, the minus iron, and the minus zinc cultures are practically indistinguishable in appearance, yield, and final pH.

That chemical precipitation of sulphur in a form relatively unavailable to the fungus is the underlying cause of the action of barium is also borne out by the evident harmlessness of barium in the low sulphur and organic sulphur cultures. The high yields with organic sulphur present in a form not precipitated by barium would indicate moreover that sulphur, once it has penetrated the cells, can be utilized by the fungus despite the presence of barium in the nutrient solution. The progressive decreases in yield and increases in pH at harvest with an increasing concentration of barium salt when sulphur is supplied as sulphide would indicate that growth is due to traces of sulphate.

The absence of any uniformity in response to the various barium salts in McHargue's experiments (3) would seem to eliminate nutrition, partial substitution, and ion toxicity as the factors responsible for the increases reported with green plants. That is to say, the increased growth is in all probability due to an improvement in the physicochemical properties of the nutrient solution, and only secondarily to any action on the plants. Sufficient evidence is not available, however, to determine the specific cause, such as increased availability of magnesium, favorable modification in concentration of one or more of the nutrient ions, or complex salt formation, etc. There is every reason to believe that many cations and anions of the unessential elements may function to aid the organism through adjustment of the physicochemical properties of the environment without entering directly into the metabolism of the organism. Such for example is the case cited in this paper in which an increase in growth is accomplished through addition of barium chloride to the nutrient solution.

In nutritional studies with the plant it would seem desirable to employ where possible the optimum solution; that is to say, to uti-

lize a solution containing the necessary nutrients in amount just sufficient for maximum normal development and therefore free from nutrient deficiency or ion toxicity. Partial substitution because of nutrient deficiency also cannot be a factor in the results with the optimum solution. Partial substitution, or rather substitution, since it is *a priori* impossible wholly to replace an essential element in its functions in the protoplasm, has reference here to the actual substitution for the element incorporated into the cell structure of an unessential element or another essential element. Although the distinction has been overlooked, substitutions in the nutrient solutions having the effect of conserving or expediting the utilization of nutrients are also possible and may be referred to as "replacement." One of the ultimate effects of the interplay of substitution, replacement, ion toxicity, and physicochemical modification of the nutrient solution is to increase the optimum ranges of the individual essential inorganic constituents so that maximum growth is capable of being attained despite a marked variation in nutrient proportions and concentration (1).

With these facts in mind it must be admitted that there is more than one nutrient solution with respect to composition that may be properly referred to as optimum in that it permits of the apparently normal maximum development of the organism. The true optimum solution has reference, however, only to one capable of affording a maximum normal development with a minimum of constituents under a definitely prescribed physical environment. Its nutrient proportions are not necessarily absolute since here also substitution and replacement are feasible and may still occur to a limited extent. The true optimum solution is the minimum balanced diet required for maximum normal development. Its probable variation in absolute concentration with variation in temperature and other physical factors, moreover, does not detract from its usefulness, if experience in animal nutrition is a satisfactory criterion.

Use of an optimum solution containing a minimum of constituents possesses several advantages experimentally over that of other optimum solutions. An optimum solution composed of nutrients in uselessly excessive quantities leads to the introduction of increased quantities of the ever present impurities they contain. In the pres-

ence of an excess of nutrient compounds maximum growth occurs only because any ion toxicities due to the individual components mutually cancel or mask each other. Also there is as yet no proof that a solution physiologically balanced with respect to a single function is necessarily balanced with respect to all functions, so that the use of needlessly excessive concentrations should be avoided. Because of the decrease in ion toxicities of the individual constituents with decreasing concentration, these effects will be at a minimum, in general, in the optimum solution which is most dilute. It follows moreover that ion toxicities that may arise from depletion of the nutrient solution will also be at a minimum.

It is obvious that in the experiments herein reported, the formation of translucent and watery hyphae in the absence of sulphur is a morphological abnormality due to nutrient deficiency. Other data on hand afford proof that a deficiency of nitrogen, phosphorus, magnesium, iron, or zinc also leads to similar results. It may be concluded therefore that the morphological characteristics of the control or so-called "unstimulated," "normal," or minus zinc culture usually obtained in zinc stimulation studies are really the abnormal, whereas those of the "stimulated," or zinc culture, are actually the normal. The barium effect within the range studied is due to the removal of sulphur.

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LITERATURE CITED

1. HOAGLAND, D. R., Optimum nutrient solutions for plants. Science N.S. 56: 562–564. 1920.
2. JAVILLIER, M., Sur la substitution au zinc de divers éléments chimiques pour la culture du *Sterigmatocystis nigra*. Compt. Rend. Acad. Sci. Paris 155: 1551. 1912.
3. McHARGUE, J. S., Effects of certain compounds of barium and strontium on the growth of plants. Jour. Agr. Res. 16:183–194. 1919.
4. STEINBERG, R. A., The so-called "chemical stimulation" of *Aspergillus niger* by iron, zinc, and other heavy metal poisons. Bull. Torr. Bot. Club 61:241–248. 1934.

DAILY VARIATION IN THE SENSITIVITY OF MIMOSA WITH SPECIAL REFERENCE TO THE ACTION OF LIGHT

W. E. BURGE, G. C. WICKWIRE, AND H. J. FULLER

(WITH TWO FIGURES)

As is well known, the leguminous plant *Mimosa* responds to a variety of external stimuli with an infolding of the secondary leaflets and a fall of the main petiole. The present investigation was begun to determine what effect, if any, daylight and darkness would have on this capacity; in other words, to determine the effect of light and darkness on the sensitivity or irritability of *Mimosa* when the temperature effect was excluded by keeping this constant.

Vigorous greenhouse plants about 18 cm. tall were used. These plants were grown from seed sown in 4-inch pots in April, 1934, and were kept throughout the experiments in a well-lighted east greenhouse. Some of the experiments were performed in September and October before the onset of cold weather, others in November and December. During the latter months the greenhouse was maintained at a temperature of approximately 26°–27° C. The degree of irritability was ascertained by determining the weakest stimulus that would cause a downward movement of the main petiole. Later it is proposed to use chronaxie as a measure of irritability, instead of minimal or threshold stimulus as was done in this investigation, when a comparison of the irritability of *Mimosa* will be made with that of other plants and animal tissues. The method of stimulation was to drop cylindrical pieces of wood, 3 mm. in diameter and of various lengths, through a glass tube 25 cm. long with a bore of 7 mm., so that they would strike the plant at the junction of the four primary leaflets as shown in figure 1.

One leaf of each of nine plants was selected for stimulation. At 6 o'clock in the morning, which is about daybreak at the first of December, the stimulations were carried out, beginning with the smallest weight (15 mg.) and using the other weights in rapid suc-

cession in the ascending order. In this way the weakest stimulus was determined which would cause a drop of the leaves. Three hours later, at 9 A.M., the same procedure was repeated, and again at noon, 3 P.M., 6 P.M., 9 P.M., midnight, 3 A.M., and 6 A.M. the next day.

The continuous line curve shown in figure 2 was constructed from the average of the data obtained from the nine plants. The weights in milligrams of the cylindrical pieces of wood that were found to be minimal or threshold stimuli are the ordinates while the times of day these weights were effective in producing a drop of the leaves are the abscissas. It will be seen that at 6 A.M. a weight of 68 mg. was required to produce a drop of the leaves while at 9 A M. a weight of 35 mg. was sufficient. This response is taken to mean that irritability of the leaves had

FIG 1 —Method of procedure in dropping weights through tube to stimulate the plant, *Mimosa*

risen between 6 and 9 o'clock in the morning. The fact that relatively weak stimuli evoked a response of the leaves throughout the remainder of the day is likewise interpreted to mean that irritability remained relatively high during the day. It may be seen further (fig. 2) that as the evening came on, stronger stimuli were required to evoke a response, until the following morning at

6 A.M. when a 65 mg. weight was required. Apparently irritability gradually decreased during the night to the original low ebb around 6 A.M., approximately the same as it was on the preceding morning when the experiment was begun.

It should be mentioned in this connection that a considerable difference was found in the degree of irritability of the nine plants used, but all of them showed the same abrupt rise in irritability during the first part of the morning, which remained high during the day and fell gradually at night.
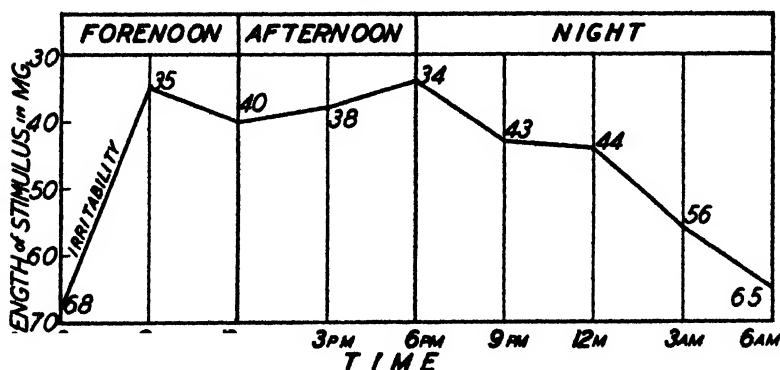
Fig. 2.—Curve illustrating that the irritability of *Mimosa* rises rapidly during the first part of the morning, remains high during the day, and falls gradually during the night.

This increase in irritability suggested that light might be responsible, and in order to determine whether this was true, two series of experiments were carried out. In one series the plants were illuminated continuously for 24 hours with a 300 watt Mazda lamp at a distance of 60 cm., and in the other series the plants were kept in darkness for a similar period of time. It was found that the irritability of the plants in the light remained at the high daytime level while the irritability of these same plants when kept in darkness remained at the low nighttime level. When the plants that had been kept in the dark were transferred to the light the irritability rose and remained at the high daytime level, and when the plants that had been kept in the light were transferred to darkness, the irritability dropped and remained at the low nighttime level.

These observations are directly contradictory to the results reported in the literature. WALLACE (7) observed that the irritability of *Mimosa* was highest around 5 o'clock in the morning, a time when we found it was lowest; that it diminished during the day when we found it was increased, and increased during the night when we found it was diminished. A possible explanation for this contradiction may be found in the different criterion used for measuring irritability. WALLACE, like other investigators in this field, used extent of movement (angle of fall) of the leaf as a measure of irritability while we used the minimal or threshold stimulus. Hence it would seem to be in order at this juncture to look into the merits of the criteria used.

The drop of the leaf of *Mimosa*, when stimulated, is due to the loss of water from the parenchymatous cells on the lower side of the pulvinus. What the stimulus does is to cause these cells to give up water, as a result of which the petiole falls. Hence it seems that the degree of drop of the leaf of *Mimosa* is an index to water loss rather than to irritability. Irritability in this case is the *capacity* of the leaf to respond to the stimulus, while water interchange is connected with another property, namely turgidity, which is involved in the actual *response*. In the works of WALLACE and others, the degree of response, not the capacity to respond, has been measured and has been (erroneously, it seems to the writers) taken as the criterion of the capacity to respond.

WALLACE (6) also used the extent of movement of the leaf of *Mimosa* as a measure of irritability in his study on the influence of chloroform, ether, nitrous oxide, ethylene, various other chemicals, temperature, age, etc. BOSE (1) and the earlier workers on *Mimosa* used the extent of movement of the petiole as a measure of irritability, although in most cases quantitative measurements are rare. CORRENS (3), in his work on the relation of free oxygen to responses in *Mimosa*, used the fall of the main petiole and the degree of folding of secondary leaflets as the indices of sensitivity. PRINGSHEIM (5) writes, "External factors which lower the sensitivity [of *Mimosa*] without killing the plant, can affect the plant in such a way that a given contact stimulus may initiate a response of only half the normal extent." MACDOUGAL (4) states, "The angles [of petioles with

the main stem] should be measured exactly with a protractor," in a discussion on irritability and response in *Mimosa*.

It is recognized that the extent of muscular contraction is not a measure of irritability but of contractility, and that the lowering of temperature of a muscle decreases its contractility but increases its irritability. Likewise, the suspending of a weight from a leaf of *Mimosa* decreases the extent of movement of the leaf but does not decrease its irritability (2).

A priori, it is difficult to understand why the irritability of *Mimosa*, or the susceptibility of this plant to the action of stimuli, should be at its lowest ebb during the day, a time when the activity of the plant is highest and it is being most intensely stimulated, and irritability highest at night, a time when the plant is being least intensely stimulated. It would seem that the reverse should be true, as is found to be the case when threshold stimulus is used as a measure of irritability rather than extent of movement.

Summary

1. The irritability of *Mimosa pudica* rises rapidly during the first part of the morning, remains high throughout the daylight hours, and falls gradually during the night to a minimum around daybreak.

2. If plants are kept under continuous illumination for 24 hours, irritability does not fall during the night period as normally occurs but remains at the high daytime level.

3. If plants are kept in the dark for 24 hours, irritability does not rise during the daytime period as normally occurs but remains at the low nighttime level.

4. It is concluded that the increase in irritability during the day is due primarily to the effect of light.

5. Other investigators have found contrary to our observations that the irritability of *Mimosa* is lowest in the daytime and highest in the night. This contradiction in results is attributed to the use by other observers of an improper criterion and method for determining irritability.

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LITERATURE CITED

1. Bose, J. C., Researches on irritability of plants. London. 1913.
2. Burge, W. E., and Wickwire, G. C., A study of irritability in the plant. Amer. Jour. Bot. Suppl. to 21: no. 10. 1. 1934.
3. Correns, C., Ueber die Abhängigkeit der Reizerscheinungen höherer Pflanzen von der Gegenwart freien Sauerstoffes. Flora 75:87–151. 1892.
4. Macdougal, D. T., Textbook of plant physiology. p. 13. New York. 1901.
5. Pringsheim, E. G., Die Reizbewegungen der Pflanzen. p. 226. Berlin. 1912.
6. Wallace, R. H., Studies on the sensitivity of *Mimosa pudica*. II. The effect of animal anesthetics and certain other compounds upon seismonic sensitivity. Amer. Jour. Bot. 18:215–235. 1931.
7. ———, Studies on the sensitivity of *Mimosa pudica*. III. The effect of temperature, humidity, and certain other factors upon seismonic sensitivity. Amer. Jour. Bot. 18:288–307. 1931.

DOUBLE INTERLOCKING OF BIVALENT CHROMOSOMES IN LILIUM ELEGANS[1]

J. M. BEAL

(WITH TWO FIGURES)

Interlocking of bivalent chromosomes during meiosis appears to be of somewhat frequent occurrence in both plants and animals. SAX and ANDERSON (5) reviewed the cases known up to 1934, and discussed them with relation to their bearing upon the classical and partial chiasmatypy theories. All the cases then known were of the single type and the great majority of these showed proximal interlocking. The high frequency of proximal interlocking, together with the infrequency of distal interlocking, was used as an argument in support of the classical theory of chiasmatypy. Furthermore, at this time interlocking was known only in genera in which segmental interchange is commonly found. Other cases have since been found in genera in which segmental interchange is unknown. In an analysis of ten metaphase bivalents in *Lilium* showing single interlocking, MATHER (4) found three examples of interlocking in the attachment or proximal loop, five in the loop next the attachment loop, and two in the loop next but one to the attachment loop. As MATHER points out, since interlocking is dependent upon pairing between the homologous chromosomes, the type of pairing is very important. Pairing may begin (1) at random along the length of the chromosomes when the nucleus is not polarized, or (2) at the ends when the nucleus shows polarization. In *Tradescantia*, in which interlocking is common (5), the nucleus is polarized. Pairing thus begins at the ends of the chromosomes and chiasma formation occurs early there. This appears to inhibit chiasma formation in the median region of the paired chromosomes. ANDERSON and SAX (1) now state that this interpretation of chromosome pairing explains the prevalence of

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

proximal interlocking in *Tradescantia* and invalidates the data pre-
viously cited (5) as evidence in favor of the classical theory.

There is no evidence of polarization in *Lilium*, hence pairing and
interlocking (when it occurs) should be at random. And since there
is little terminalization of chiasmata in *Lilium*, the position of the
interlocked chromosomes should show little change during the later
prophase stages. This assumption is supported by the observations
of MATHER on the cases of single interlocking in *Lilium*, as well as by
the known cases of double interlocking. Double interlocking, on the
other hand, appears to be extremely rare, since only two examples
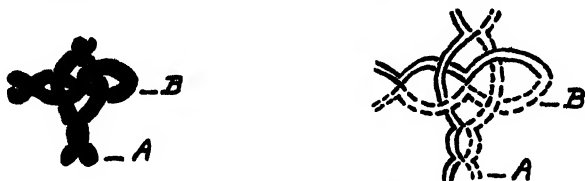
Figs. 1, 2.—Fig. 1 (left), double interlocking between the two bivalents of *Lilium
elegans*. Both chromosomes of bivalent A are interlocked in two adjacent internodes of
bivalent B. ×ca.2400. Fig. 2 (right), probable chromatid relations in the two inter-
locked bivalents of figure 1, based on the partial chiasmatypy theory.

have been reported previously. The first of these was described by
MATHER (3) for *Lilium regale* and the second by UPCOTT (6) for
Eremurus. A third case can now be recorded.

During the examination of smear preparations of the pollen
mother cells of *Lilium elegans*, fixed in Flemming's medium solution
and stained in accordance with Newton's gentian violet-iodine
method, a single example of double interlocking was observed (fig.
1). Both homologous chromosomes of bivalent A are interlocked be-
tween the two chromosomes of bivalent B, one chromosome of A
being interlocked in the attachment or proximal loop of B and the
other in the loop next the attachment loop. A chiasma is present in
bivalent B between the two loops, and each of the interlocked biva-
lents shows a total of three chiasmata.

During the zygotene-pachytene pairing both chromosomes of
bivalent A must have been caught at some distance apart between
the two pairing chromosomes of bivalent B. The formation of a
chiasma by the two chromosomes of bivalent B between the two
chromosomes of bivalent A effectively prevented the latter two

chromosomes coming together at this point. Figure 2 shows an analysis of the probable chromatid relations of figure 1.

MATHER (4), in a study of several species of *Lilium*, has shown that there is little reduction in the number of chiasmata from diplotene to metaphase. *Lilium elegans* shows little reduction, and it is highly improbable that two nodes or two chiasmata had been present during diplotene in bivalent B between the two interlocked chromosomes of bivalent A. DARLINGTON (2) has pointed out that paired chromosomes remain paired at diplotene by both chiasmata and relational coiling. It is often difficult to distinguish between these two conditions during early diplotene and no doubt relational coiling has often been confused with chiasmata, especially in those cases where chiasmata are formed at random. In *Fritillaria* species in which chiasmata are localized, and are thus more easily recognized, there is practically no reduction in the number of chiasmata between diplotene and metaphase (2). In *Lilium*, with random chiasmata, the apparent reduction in the number of chiasmata may be explained on the basis of the disappearance of relational coiling rather than as a result of terminalization or breaking of chiasmata, since there is slight terminalization and little or probably no breaking of chiasmata.

It is therefore highly probable that a single chiasma was formed in bivalent B between the interlocked chromosomes of bivalent A and that no reduction or breaking of chiasmata has occurred. If this assumption is true, then the arrangement of the chromatids as shown in figure 2 must be true and adds strong support to the partial chiasmatypy theory of crossing-over.

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LITERATURE CITED

1. ANDERSON, EDGAR, and SAX, KARL, A cytological monograph of the American species of *Tradescantia*. BOT. GAZ. 97: 433–476. 1936.
2. DARLINGTON, C. D., The internal mechanics of the chromosomes. I, II, III. Proc. Roy. Soc. Series B. 118:33–96. 1935.
3. MATHER, K., Interlocking as a demonstration of the occurrence of genetical crossing-over during chiasma formation. Amer. Nat. 67:476–479. 1933.
4. ———, Meiosis in *Lilium*. Cytologia 6:354–380. 1935.
5. SAX, KARL, and ANDERSON, EDGAR, Interlocking of bivalent chromosomes in *Tradescantia*. Genetics 19:157–166. 1934.
6. UPCOTT, M. B., The origin and behavior of chiasmata. XII. *Eremurus*. Cytologia 6: (in Press). 1935.

CURRENT LITERATURE

Pollen Grains: Their Structure, Identification, and Significance in Science and Medicine. By R. P. WODEHOUSE. New York: McGraw-Hill, 1935. Pp. xvi+574. Illustrated. $6.00.

The first comprehensive study of pollen grains to appear in the English language is welcomed by pollen analysts and hay fever specialists. Morphologists and taxonomists may also consult the work with profit since, in addition to that drawn from the author's earlier papers, much new material is included to prove the thesis that "in no other part of the plant are to be found packed in so small a space so many readily available phylogenetic characters." The approach is therefore on the basis of comparative morphology.

The volume falls into two sections. Part I includes the following chapters: historical review; methods of collecting pollen in large amounts; preparation of pollen for microscopic examination; pollen statistics: a botanical and geological research method by G. ERDTMAN; atmospheric pollen (at Yonkers, New York); hay fever; and pollen grain characters. Eighty-five pages are taken up by the historical review, and the contributions of VON MOHL, FRITZSCHE, and HUGO FISCHER are described in detail.

The chapter on pollen grain characters forms the necessary morphological background for part II. The author points out that "while heredity or phylogeny tends to dominate the basic form of pollen grains, the internal environment tends to control the number and arrangement of their germinal furrows and pores, and the external environment (wind *versus* insect pollination) tends to modify their sculpturing." His treatment of the last point seems somewhat teleological throughout the book. The various furrow patterns are subjected to mathematical analysis and most of them are seen to fall into the trischistoclasic system.

The section on classification is headed with a key to 41 angiosperm families or their representatives, and to the seven orders of gymnosperms. The text material which fills the remainder of the volume presents specific, generic, and family descriptions, and keys to certain genera and species. Thirty-five genera of living gymnosperms, representative fossil forms, and 31 angiosperm families are treated. These, according to the author, "fit together, quite naturally, in a sequence corresponding, for the most part, to the system of ENGLER and PRANTL." The evidence from pollen morphology for this sequence of families in the dicotyledons seems inconclusive, since the basic plan is common throughout, and many of its variations are repeated in families which are widely separated in the Englerian system. The major departure from this system involves

the position of the Magnoliaceae as the primitive family. According to WODE-HOUSE, the members of the Magnolieae show single-furrowed pollen grains which are virtually indistinguishable from those of the Bennettitales. He derives the tricolpate grain of nearly all other dicotyledons from the primitive monocolpate grain through the anomalous pollen of *Schizandra* and *Kadsura*. These two genera exhibit "side by side, the principal features of the triradiate pteridophyte spore, the monocolpate gymnosperm pollen grain, and the tri-schistoclasic dicotyledonous pollen grain."

Other phylogenetic interpretations based on pollen characters include the following ideas. The Nymphaeaceae, the monocotyledons, and the six tribes of the Coniferales, in addition to the Magnoliaceae, may be regarded as coordinate genetic lines. These types inherited the broad open type of furrow characteristic of the Cycadales, *Ginkgo*, and the Bennettitales. Further pollen grain evolution along these genetic lines has centered in means of modifying, protecting, or eliminating this furrow. With regard to the evolution within the Coniferales, WODEHOUSE's interpretations are suggestive. He believes that the Taxodineae and the Cupressineae originated from the cordaitalean stock independently of the Abietineae, and that the Taxineae are closely related to them. The Podocarpineae are most closely related to the Araucarineae, although *Araucaria* and *Agathis* probably represent the end of a distinct line of development. His treatment of the monocotyledons is very brief, although the pollen of the Palmaceae is thought to be the most primitive of the forms discussed. In spite of this, he places this family after the Typhaceae and Naiadaceae in his sequence of families.

As one might expect in a work of this size, the book is not free from error. Thus one finds that *Welwitschia* exists in southeast Africa, and that beech pollen has not been recorded from bog deposits in America. *Digitaria* is placed in the generic sequence of the Festuceae, and synonyms are sometimes used indiscriminately. One may also consult the bibliography in vain for certain citations in the text.

But these points are minor. The beauty and clearness of the numerous plate and text figures, the lucid and concise descriptions, and the suggestive phylogenetic interpretations make this book a volume which many botanists and physicians will wish for their libraries.—C. E. OLMSTED.

Vergleichende Morphologie der höheren Pflanzen. By WILHELM TROLL. Vol. I. Part I. Vegetationsorgane. Berlin: Gebrüder Borntraeger, 1935. Pp. 172. With 104 illustrations.

The initial section of the first volume of a comprehensive German work on the comparative morphology of the higher plants has recently been published. The author, Dr. WILHELM TROLL, professor of botany at Martin Luther University, Halle, Wittenberg, has outlined the scope of the complete work in the foreword. It will consist of three volumes of several numbers each, which will

be published at regular intervals. The first volume, in addition to the introductory chapters, will be devoted to the morphology of the vegetative organs while the remaining two will deal with the comparative morphology of the inflorescence, including flower, fruit, and seed development. Some consideration will be given to the vegetative organs of the pteridophytes, but the major emphasis will be upon the seed plants.

Part I of the first volume includes an introductory section, and the first main section of the work which is concerned with the gross morphology of the vegetative organs of the seed plant. In the introductory passages general biological concepts are discussed, including sections on homology, analogy, and convergence, teratology, organography, developmental and experimental morphology, and phylogeny. The main section of 25 chapters deals with the gross morphology of the higher plants, and selected examples of common plants are used as illustrative material. Representative chapters are those on the seed, embryo, and development of the root of the radish; development of the leaf of the pea; seedling and shoot development in corn; the development of the onion; tuber formation in the potato; and others dealing with buds, bud scales, and bud development, phyllotaxy, seed germination, bulb formation, and root and shoot development.

The selection of common native and economic plants as examples and for illustrative purposes is most desirable and will undoubtedly add to the value of the work. It is refreshing to find this tendency to use well known plants appearing in morphological studies; they have too long been devoted to elaborate investigations of remote and little known plants.

An accurate evaluation of this work must await the appearance of succeeding sections as the initial one is introductory and general in character. The real worth of the forthcoming parts will depend in large measure upon the nature of the detailed morphology included and the extent to which dynamic and developmental phases of plant anatomy are stressed.—H. E. HAYWARD.

Protoplasm. By WILLIAM SEIFRIZ. New York: McGraw-Hill, 1936. Pp. x+ 583. $6.00.

About a century ago biologists began to recognize the slimy material which they observed within the cells of organisms as living substance. The term protoplasm to designate the fundamental living material was first used a little over ninety years ago, but did not become popular until VON MOHL gave it prominence in connection with his investigations in 1846. Many of the great biological speculations have developed from the contemplation of the ultimate nature and intimate structure of protoplasm and from attempts to explain the transmission of hereditary qualities from generation to generation. Such units as biogens, bioblasts, gemmules, pangens, plastidules, biophores, genes, micellae, etc., have been postulated by the speculative biological philosophers of the nineteenth century. But aside from philosophical speculations,

a vast store of information has been accumulated by investigators who have taken protoplasm as a concrete but variable material to be studied by every means known to man. Physico-chemical methods and studies of matter in the colloidal state have thrown a flood of light upon the behavior and nature of this most remarkable substance in the universe.

The important advances which these investigations have made possible are summarized by SEIFRIZ in a book that students of biology will welcome. The principal emphasis of the volume is placed upon the physical and physico-chemical properties of protoplasm. The early chapters consider the general properties of living protoplasm, its organization into cells, and methods of studying its properties and behavior, such as micrurgy and tissue culture. The colloidal properties and structure of protoplasm naturally receive a great deal of attention in chapters on the colloidal state, emulsions, hydrophilic sols and gels, and in the consideration of such properties as surface tension, adsorption, osmosis, imbibition, viscosity, elasticity, and permeability to water, ions, and molecules.

In later chapters acidity, electrophysiology, electrokinetic phenomena, and the influences of radiant energy are summarized. These are followed by a consideration of the role of water, salts, and the food materials, carbohydrates, fats, and proteins. A brief chapter covers the regulatory mechanisms, enzymes, hormones, vitamins, pigments, and other regulatory substances of animal and plant protoplasms. In places the information will seem meager, but the author could not have kept himself within the limits usually imposed if the treatment were adequate at all points. A final chapter deals with the origin of living matter, a problem that will probably remain with biologists for many years as a challenge to more fundamental investigation. In connection with the reference to BENJAMIN MOORE, one misses consideration of the recent papers of FRANCIS which continue the ideas that MOORE first expressed and illustrate them with many contributory observations. The bibliography of choice selections for collateral reading on the various chapters will be helpful to students.

The book is written primarily for students, is not too technical, and is an interesting and valuable contribution which will bring pleasure and stimulation to those who are fortunate enough to have the privilege of reading and digesting the abundance of information presented.—C. A. SHULL.

Ugressfrø (Unkrautsamen, Weed Seeds). By EMIL KORSMO. Oslo, Norway: Grøndahl and Sons Boktrykkeri, 1935. Illustrated.

This recent book by EMIL KORSMO is destined to aid materially in weed and seed investigations. It contains a wealth of material, representing the culminating effort of a lifetime devoted to a subject that in most countries has been somewhat neglected.

The book contains descriptions and illustrations of 306 plant species characterized as weeds. The species included are those found commonly in east,

west, central and north Europe, and less commonly in south Europe and North America. There are 34 full-page colored plates, each with illustrations of nine species. Each illustration in clear detail includes all or part of the inflorescence; a fruit depicting the method of dehiscence (for dehiscent fruits); natural and enlarged views of the seed; and finally transverse and longitudinal sections of the seed showing the position and relative size or thickness of the endosperm, embryo, and seed coats. The illustrations are all in natural colors, although it must be recognized that the colors exhibited by many species are not the same in every geographical area. Preceding each plate is a description in Norwegian, German, and English of each species figured. The description covers the type of inflorescence, kind of fruit (silicle, achene, capsule, etc.), shape and size of seed, color and appearance of the seed coat; and the distribution, common habitat, and methods of seed dissemination. The species are arranged according to what the author calls life forms, designated by symbols (annuals, winter annuals, biennials to perennials, and perennials with rootstocks). Within each group the species are arranged in families. From the standpoint of the teacher or the taxonomist, the major grouping is somewhat unsatisfactory, but the aim of the author has been to serve the agriculturist as well as the botanist, recognizing at the same time that the life history of a given species is affected by geographical distribution. The index lists alphabetically the botanical names of all the species together with their common names, in eleven languages. The book will doubtless find a place on the reference shelves of those who teach courses dealing with weeds and seeds, and will also serve as a material aid to the investigator and the seed analyst.—R. H. PORTER and T. E. MELHUS.

Tree Flowers of Forest, Park, and Street. By WALTER E. ROGERS. Appleton, Wisconsin: Walter E. Rogers, Lawrence College, 1935. Pp. 500. Illustrated.

This is the only work of its kind known to the reviewer. By means of many handsome plates, done in a "duotone green," the flowers of our common trees are shown on a large scale of magnification and with a fidelity to nature seldom equaled in the whole domain of floral literature. The book "is intended to be and is chiefly pictorial," therefore the textual material is mostly of a supplementary and simplified nature. By means of many skillfully drawn silhouettes, the author portrays in an ingenious way the deciduous trees in their winter state of defoliation. Art teachers and botany teachers will undoubtedly join in praise of this outstanding feature, since it affords a most effective way of imparting to students instantly a realization of some of the trees' major characteristics. Students of conventional design will find an abundance of source material on which to draw for application to the needs encountered in the practical arts. Much of this material has hitherto escaped the notice of all but botanists, for the very reason that it was of diminutive size.

The mechanical features are of the highest order and combine to make a truly sumptuous volume, the publication of which represents a landmark of achievement for the science of botany in America.—E. E. SHERFF.

Die Pilze Mitteleuropas. Editorship of H. KNIEP, P. CLAUSSEN, and J. BASZ. Leipzig: Werner Klinghardt, 1935.

Die Pilze Mitteleuropas has been appearing in serial form. The fifteenth issue of volume I has appeared, containing the Boletaceae by KALLENBACH. In the meantime volume II has been begun with three issues. These are devoted to Tremellineae by NEUHOFF, and Lactari by KNAUTH and NEUHOFF. The new issues continue on the high level previously established. The publisher announces a reduction of 25 per cent in the price to foreign subscribers.—G. K. K. LINK.

Die Pflanzenzelle—Vorlesungen über normale und pathologische Zytomorphologie und Zytogenese. By E. KÜSTER. Jena: Fischer, 1935. Pp. xii+672. Illustrated.

This volume by KÜSTER is a notable contribution not only to botany but to other biologic sciences as well. It is a synthesis of a vast literature by one who has enriched it significantly through several decades. Its value is enhanced by critical evaluations of present trends and by suggestions of promising investigations. One is also permitted stimulating glimpses of the author's view on theoretical biology. The volume deals with both normal and abnormal plant cell morphology and cytogenesis. These correlative aspects of cellular life are so well integrated that their essential unity is apparent at all times. For this reason the volume will prove interesting and useful both to cytologists and to pathologists in the botanical and zoological fields.

Inclusion of the normal and anomalous in one volume should do much to break down the artificial barriers that have tended, since the last quarter of the 19th century, to segregate as independent biological provinces the normal and healthy on the one hand and the anomalous and pathic on the other. This separation, warranted at one stage in biology because it formulated and focused attention on specific problems, has outlived its usefulness. There has always been difficulty and confusion in delimitation of the pathologic. Some of the difficulties are presented in the introductory discussions of KÜSTER's well known earlier volumes, Pathologische Pflanzenanatomie and Pathologie der Pflanzenzelle. In general the pathologic has been equated with (a) the statistically abnormal, (b) the abnormally induced, (c) harmful abnormality, (d) destruction and the dysfunctional, that is, harmful events (injuries) at or between the surfaces of organisms and their parts, irrespective of the normality or abnormality of their occurrence or causation.

KÜSTER defines the field of the pathologic as comprising all events due to anomalous conditions and agents, both internal and external. The pathologic accordingly includes all experimentally induced effects.

To consider as pathologic any event due to statistically abnormal causes is essentially the same as defining it as a statistically anomalous event. The discovery that variation is a pervasive characteristic of living things seems to indicate that this criterion of the pathologic is too inclusive.

Considered from another angle the criterion is too exclusive. Unreserved exclusion of the statistically normal from the field of the pathologic bars many events that constitute either destruction (necrosis is a special case) or functional impairment (failure to adapt and to survive is an extreme case) at one or more levels of biologic organization. In the reviewer's opinion, if only one criterion for the pathologic is sought, it should be the criterion of harm or injury in the broadest sense of the term. Thus any event is pathologic that actually or potentially destroys or functionally impairs any given system as a whole, or any part of such system. Strictly speaking, a harmful event (injury) plus the train of reactions incited by it constitutes the complex known as disease. Injury and reaction to injury accordingly constitute the center of the concept pathologic, while the anomalous is its fringe. This definition includes both normal and abnormal injuries and injury reactions in the field of the pathologic.

KÜSTER unreservedly accepts as pathologic all events which stand in a causal relation to death. Nevertheless he chooses to designate them physiologic if they are statistically normal. He speaks of physiologic degeneration, destruction, death, etc. Granted that natural, normal, regular, and similar statistical concepts are some of the ancient meanings of the physiologic, is it not advisable for the sake of greater precision to abandon this usage together with that other misnomer, physiologic disease, which is so frequently and confusedly used by botanists? Formulation of the physiologic-pathologic antithesis in causal terms has done much to engross plant and animal pathologists in consideration of causal agents, especially parasites, with attendant neglect of the material or functional disturbances of the affected system. In most American botanical textbooks the concepts abnormal, pathologic, and disease are equated and introduced with and limited to discussions of parasitic bacteria and fungi. If physiologic and pathologic are to be used as antithetic terms, is it not preferable to give them a teleologic content, for which there is ample precedent, and designate as physiologic those events which are functional with reference to the system under consideration, including keeping itself adapted and alive, and as pathologic the destructive and dysfunctional ones?

The subject matter of the volume is treated in seven chapters: Protoplasm, nucleus, plastids, vacuoles, starch, crystals and dead inclusion bodies, wall, and cytogenesis. By choice caryology is less completely treated than the other topics. In each chapter discussion of the abnormal and pathologic immediately follows presentation of the normal and healthy.

Protoplasm (cytoplasm in the sense of STRASBURGER), nucleus, and plastids are recognized as three forms of living matter of cells, while vacuoles, inclusion bodies such as starch, and the wall are considered dead products of the living substance. There is no hint as to whether genes are considered living or non-living; or as antecedents or products of other cellular substances. The dead parts of cells are recognized as constituents of the living continuum known as the protoplast. KÜSTER accepts the proposition that protoplasm is fluid and characterized by sub-microscopic structure. He points out that in the last analysis

all protoplastic activities, including self-regulation, are in some way based on and involve this sub-microscopic structure. Its irreversible destruction is the essence of partial or total protoplastic necrosis. Lesser material and functional injuries, including loss of self-regulation, undoubtedly involve alteration of this structure. In discussing the problem of plasmodesmata, KÜSTER takes a very guarded stand and points out that these structures are not essential for correlation between protoplasts. The proposition that chondriosomes are stages in development of plastids is rejected. KÜSTER does not consider convincing the evidence advanced for considering them living substances.

The chapter on cytogenesis includes an interesting discussion of the characteristics of living substances. KÜSTER emphasizes assimilative activity and division, but points out that possibly respiratory activity may prove to be a basic criterion of living substance. This chapter also includes a discussion of problems of individuality of cells and of multicellular wholes, of correlation, of senescence, and of death.

KÜSTER subscribes to the proposition that the living substance of unicellulars is potentially immortal provided it is given opportunity to grow and to divide, and is not poisoned by its environment. He does not define as death the destruction of individuality of a protoplast incident to its division. Since most protoplasts of multicellular organisms soon lose the opportunity for division, they become so senescent that death is unavoidable. Senescence is assumed to proceed more or less rapidly in every protoplast and is attributed to hysteretic changes in the protoplastic colloids.   ·

These and other discussions in the chapter imply that the concepts health and disease not only are relative but correlative as well. Instead of asking, "Is this living protoplast healthy or diseased?" one should ask, "To what degree is it healthy and pathic?" KÜSTER's statement, "The physiology of senescence and the pathology of the cell are not separable" is a modern version of ARISTOTLE's statement, "Indeed we may rightly call disease an acquired old age, old age a natural disease," and of CLAUDE BERNARD's aphorism, "The healthy are really invalids unaware of their illness."

While reading this excellent volume, the reviewer repeatedly wished that the time might soon come when biologists would make some agreement as to usage of such basic concepts as cell, protoplasm, wall, membrane, and the like.

Each chapter closes with an excellent bibliography. An authors' and a general index close the volume. The style is easy and clear and the material is well organized. The volume, copiously and excellently illustrated, is printed on good paper and well composed. It is a credit to the author and publisher, and a rich contribution to biology.—G. K. K. LINK.

THE
BOTANICAL GAZETTE

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ECOLOGICAL STUDIES IN THE LOWER ILLINOIS RIVER VALLEY

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 470

LEWIS M. TURNER[1]

(WITH ONE FIGURE)

Introduction

The central section of Illinois lies between 39° and 41° north latitude. Ecological studies were made adjacent to the Illinois River, in the southwestern part of this section, in Jersey, Calhoun, Greene, and southern Pike counties.

Several factors have contributed to the disturbance of the native flora on the floodplain or bottomland along the Illinois River in the region of this investigation. The two lower dams (10) in the river have raised the water level of the lakes. Discharge of water from the Chicago drainage canal (1, 10) has increased the average water level in this section 2.5–3 feet. Levees (10) have been constructed in many places for the purpose of reclaiming bottom lands for agriculture. The continuity of these levee areas is occasionally interrupted either because of lack of construction or because of the entry of tributaries into the river. Where these gaps occur the land is subject to overflow, and hence is generally not cultivated. These are the only areas representing the prelevee era of the valley, and here the flora has, to a large extent, retained its primitive condition. Such primitive flora was observed at the mouth of Apple Creek.

Physiography and geology

The Illinois River (2, 9), formed by the junction of the Des Plaines and Kankakee rivers in northeastern Illinois, flows 273 miles across the state in a southwesterly direction and joins the Mississippi River at Grafton. The fall of the river from LaSalle to its mouth, a distance of 223 miles, is only 33 feet (1). Its floodplain or bottomland is bounded by bluffs or hills, and it varies from 1.5 to 7 miles in width, averaging about 4 miles within the range of this study.

The bottomland of the lower valley of the Illinois River has been filled to a greater depth than its upper valley (1). Here the immediate banks of the stream are high, and although the lakes are smaller and fewer than in the upper valley, many of them lie 10 feet or more above the river when the water is low.

The lakes of the floodplain are not of the oxbow type. They have been formed by the impounding of water brought about by the differential deposition of silt, and by the formation of natural levees. These lakes lie, for the most part, in the lower backwater areas of the floodplain. They occur both in the forest and in the wet prairie. The lakes have been classified as permanent and temporary. Where there are smaller exposed surfaces of water these areas are called ponds or sloughs. The lakes receive their water supply from springs or small tributaries to the river. The permanent lakes are deep and poorly drained. The temporary lakes are made shallow by frequent deposits of silt. They become dry during part of the year, except in extremely wet years. During the exceedingly dry year of 1931 some of the "permanent" lakes also became dry.

In the region studied, the rocks (9) immediately underlying the upper soil layers of loess and loam are Mississippian limestone, containing some cherty and oolitic material and overlying shale of the same epoch. The bluffs at the sides of the river valley, throughout the area, have this same composition, with the exception of southern Calhoun County where there are small outcrops of Devonian and Silurian limestone overlying thicker beds of Ordovician (Richmond) limestone and shale. The section west of the Illinois River comprising Calhoun County and the southernmost part of Pike County were unglaciated (7, 8).

Ecological factors

CLIMATE

The physiographic features of central Illinois have little effect upon its climate (**14**). This section lies in the path of the main storm tracks that cross the country. Especially in´winter these cause marked changes in the weather. The climatological data here given are from weather bureau stations in central Illinois to 1930, inclusive.

TEMPERATURE.—Central Illinois has a mean annual temperature of 52.6° F. The mean annual temperature in January, the coldest month, is 27.0°; in July, the warmest month, 76.4°. "The average minimum temperature for January is 18.4°; the average minimum temperature for July is 88.5°" (**13**). The crop-growing season averages 178 days.

PRECIPITATION.—The average annual rainfall is 36.65 inches with an annual range of 32.83 to 40.74 inches. Summer rainfall is sufficient to meet the requirement of the vegetation, although occasional droughts may occur. Most of the rainfall is of the local shower type. The snowfall averages 20.3 inches annually.

AIR MOVEMENT.—The prevailing winds in winter are from the northwest; during the summer they are from a southerly direction. Over limited areas occur occasional local squalls, and sometimes tornadoes, which cause great destruction.

SOILS

The present soils were derived from the residual material of the unglaciated area, glacial drift, and loess or windblown glacial material. Soils and their formation were studied particularly in Pike (**7**) and Calhoun (**8**) counties. Three topographical areas, each with its soil types, occur in this region: the upland, the bluff, and the bottomland.

UPLAND

The upland is characterized by two major soils, the upland prairie and the upland timber.

UPLAND PRAIRIE SOILS.—Because of the large quantity of roots of prairie grasses in the upland prairie soils, they are the richest in organic matter of all the soils in this region. These soil areas are rather

limited in extent. There is evidence that trees have recently grown on small areas of the prairie. Four types of prairie soils are found. The brown silt loam, although occupying little territory, has fair drainage and is the most extensive and fertile of the upland soils. Here is found the best assemblage of relic prairie plants. The sticky black clay loam (gumbo) is found on the poorly drained areas. The variation in content of organic matter indicates that the brown-gray silt loam on tight clay may have been timbered. It usually occurs between the timbered areas and the brown silt loam. The gray silt loam on tight clay occurs on level to slightly undulating topography. Because of the tight clay subsoil, it has poor drainage. It also contains the least organic matter of all the prairie soils.

UPLAND TIMBER SOILS.—The upland timber soils have a lower content of organic matter than the prairie soils. Yellow silt loam and yellow-gray silt loam, both derived from loess, are the principal upland timber soil types inland in Pike County (7). They are found on the somewhat level ridges of the rolling hills and on places badly broken by erosion. These broken areas are covered by plants of the oak-hickory association.

On the flat tops of the ridges and occupying only a small area is a light gray silt loam which is friable and porous. A layer of clay which is almost impervious to both air and water underlies this soil. This is a poorly drained but not a swampy soil.

The upland adjoining the Illinois River consists of a yellow-gray fine sandy loam on the less rolling land, and a yellow fine sandy loam on the hilly and very rolling, badly broken land of the deep loess areas. It is very pervious and hence has good drainage. The coarse sandy phases of this soil are found nearest the bluff.

The Calhoun County soil report (8) refers to the two soils just mentioned for Pike County as brownish yellow-gray silt loam and eroded silt loam. These are the only upland soils occurring in Calhoun County. The brownish yellow-gray silt loam increases in area toward the southeastern end of the county. All of the upland soils of this county were developed under a forest cover. They are derived from loess which overlies rock in most of the county. Almost no soil profile has been developed. It is fairly well developed only in the brownish yellow-gray silt loam. Active erosion has prevented the

development of a true soil profile in most of the county. These soils are friable and have no impervious strata in their profile; hence they have good drainage. Upon the uncultivated areas of this soil were oak, hickories, and white ash.

FLOODPLAIN OR BOTTOMLAND

BOTTOMLAND SOILS.—The valleys of the tributaries and small areas of bottomland of the Illinois River contain some soil deposits derived from the older glaciations. Most of the bottomland soils are of recent origin, however, and show no profile development. This is accounted for by annual recurrence of floods.

Floods (15) covering the floodplain to depths of 5 to 15 feet were recorded in 1883, 1892, 1893, 1903, 1904, 1908, 1913, 1916, 1920, 1922, 1926, 1927, and 1929 in the region near Kampsville. Minor floods submerging the bottoms to a depth of 1 to 5 feet occur almost annually. Most floods follow melting of the ice and snow in spring. Occasional floods occur in midsummer or fall. The spring floods usually come before the foliating period and seldom have any injurious effect except that caused by alluvial deposition.

The bottomland soils of the Illinois River seem to have three primary modes of origin: soils brought down from the upland by tributaries; coarse soils deposited by overflow of the river at flood time; and soils composed of the fine material that has settled in the backwater areas at the time of overflow. The following three soil types derived from these modes of origin have been recognized.

1. Soils carried down from the uplands are deposited as alluvial fans at the mouth of the tributaries. Brownish yellow-gray silt loam deposited upon the terraces at the base of the bluffs overlies sand and gravel. It is made up of a mixture of limestone fragments, hillside wash, and a small amount of river-deposited alluvium. It is a well drained soil and subject to overflow only in times of extremely high flood water. This soil type is always populated by the talus slope-floodplain transition forest and some members of the hillside talus slope and floodplain forests. Other deposits made near the mouths of the tributaries become a brown silt loam. This is covered either by forest or by floodplain prairie association.

2. The remaining soils are those derived primarily from the soils

deposited by the Illinois River. Mixed loam is the prevailing soil type in the belt bordering the river channel and on the islands. It consists of the coarsest materials deposited by the flood water. Typical floodplain forest grows upon this soil. Upon the rock or coarse soil of the islands a soil 1 to 5–6 feet in depth has developed. On slightly higher land is a deep brown clay loam containing some coarser material. There is evidence that the lower lying parts of this area were once populated rather extensively by the *Spartina michauxiana-Panicum virgatum* association. Most of this soil is now under cultivation. On the higher parts was found the floodplain forest.

3. In the low-lying, nearly level backwater areas, where lakes, ponds, and sloughs are commonly found, the soil is a drab clay loam. This has the finest texture and is the heaviest of all bottomland soils. It has poor drainage. Floodplain forest seldom occurs on this soil, but there is found typically the *Cephalanthus-Hibiscus militaris* association with occasional willows and green ash. Sometimes *Eleocharis* dominates this habitat, and more rare is the occurrence of the *Spartina michauxiana-Panicum virgatum* association.

BLUFF

BLUFF FACE.—The perpendicular face of the limestone bluffs provides a habitat for very few plant species. Residual and windblown soils accumulate in rock crevices where only a few herbaceous seed plants can grow.

TALUS SLOPE.—The talus slopes at the base of the bluffs consist of fragments of limestone and chert of varying size covered by residual soil and the brownish yellow silt loam washed down from the upland. Upon the surface is a rich mulch of partially decayed organic matter which, as in the case of the upland soils, does not combine to a great extent with the underlying soils. Such trees as red and hard maples, red and chestnut oaks, walnut and butternut, and basswood grow on these slopes.

HYDROGEN-ION CONCENTRATION

Soil samples for the determination of pH were taken at a depth of 10 inches. The upland soils had a hydrogen-ion concentration which ranged from neutral to pH 6. In both the upland forest and the prairie, pH 6.6 was common. The lowest pH values were found in

old fields in which there had been repeated cropping and excessive leaching.

While the floodplain soils are in general circumneutral, in this region the pH ranged from 7 to 7.8, indicating a more alkaline soil. This corresponds with the pH of the yearly flood water of the river, which has consistently tested 7.8. Floodplain lakes, ponds, and ditches also had a pH of approximately 7.8. The pH of the talus slope soil was 8.

Floristics

A transect of the Illinois River valley in the region investigated shows four sections: the upland, the bluff, the transition, and the floodplain. Each of these sections was divided into plant communities.

The plants[2] observed and recorded are divided into three groups: (1) large and small trees, and shrubs of greatest frequence, studied quantitatively and enumerated in the tables; (2) woody plants of infrequent occurrence; and (3) all herbs and some woody plants occurring in spring, summer, and fall aspects. The third group of plants has been assembled in three classes: class 1, the most common plants and those frequently occupying relatively large areas; class 2, the plants occurring fairly commonly but rarely occupying areas of wide extent; class 3, plants occurring occasionally. The order of arrangement for all species is from that of greatest to that of least frequence. Casual or rare plants are not listed.

PLANT COMMUNITIES

UPLAND

UPLAND PASTURES AND OLD FIELDS.—Because of the fragmentary occurrence and difficulty in identifying the upland prairie vegetation among the upland pastures and old fields, neither a quantitative evaluation nor a study of dominance and succession was made in this community. The data assembled, however, are of value because they give a partial record of the present condition of a plant community that is rapidly disappearing.

[2] Authorities for the species listed are those given in the seventh edition of GRAY'S Manual. The species of Gramineae were identified by Mrs. AGNES CHASE of the United States Department of Agriculture. Approximately 650 species of plants were collected, and a set was given to the Department of Botany at the University of Chicago.

HERBS

Spring

Class 1

Poa pratensis	E. philadelphicus
Trifolium repens	Potentilla canadensis
Rumex acetosella	Poa annua
Capsella bursa-pastoris	P. compressa
Erigeron pulchellus	

Class 2

Melilotus alba	P. major
M. officinalis	Oxalis stricta
Stellaria media	Apocynum cannabinum
Antennaria plantaginifolia	Achillea millefolium
Medicago sativa	Verbascum blattaria
Plantago lanceolata	

Class 3

Pentstemon gracilis	Phlox divaricata
Asclepias quadrifolia	Comandra umbellata
Silene antirrhina	Monarda bradburiana
Physalis virginiana	Blephilia hirsuta
Arabis drummondi	Polygonatum commutatum
Fragaria virginiana	Scutellaria versicolor
Amorpha canescens	

Summer

Class 1

Erigeron spp.	Stellaria media
Ipomoea hederacea	Rudbeckia hirta
Verbena stricta	Plantago aristata
Euphorbia corollata	Petalostemum purpureum
Verbascum thapsus	Cirsium lanceolatum
Strophostyles helvola	Silphium integrifolium

Class 2

Agrostis hyemalis
Hypericum punctatum
Bouteloua curtipendula
Solidago radula
Sorghum halapense

Desmodium illinoense
Monarda fistulosa
Ambrosia bidentata
Phleum pratense
Asclepias tuberosa

Class 3

Daucus carota
Solanum carolinense
S. nigrum
Asclepias purpurascens
Lechea tenuifolia
Oxalis corniculata
Ruellia ciliosa
Verbena urticaefolia
Petalostemum candidum
Setaria glauca
Coreopsis tripteris
Astragalus canadensis
Phlox pilosa
Anthemis cotula

Nepeta cataria
Verbena bracteosa
Achillea millefolium
Croton capitatus
Physalis heterophylla
Psoralea tenuiflora
Kuhnia eupatorioides
Cichorium intybus
Acerates floridana
Pycnanthemum muticum
Tripsacum dactyloides
Cuphea petiolata
Panicum capillare
Pentstemon grandiflorus

Fall

Class 1

Eupatorium torreyanum
E. sessilifolium
Andropogon furcatus
Eragrostis cilianensis

Panicum agrostoides
Sporobolus asper
Andropogon scoparius
Helianthus hirsutus

Class 2

Gerardia tenuifolia
Aster anomalus
Eragrostis caroliniana
Liatris cylindracea
L. scariosa
Cassia nictitans

Aster oblongifolius
Cirsium arvense
Lespedeza capitata
Sorghastrum nutans
Solidago spp.

Class 3

Croton monanthogynus   L. virginica
Polanisia trachysperma   L. frutescens
Lespedeza repens   Elymus canadensis
L. striata   Liatris pycnostachya

SHRUBS, PERMANENT INVADERS OF OPEN AREAS

Rhus toxicodendron   Symphoricarpos orbicula-
R. canadensis   tus
R. glabra   Corylus americana
Cornus asperifolia   Sassafras variifolium
Sambucus canadensis   Rosa cinnamomea

At the beginning of spring in this prairie region a considerable number of introduced species appear, many of which are common weeds. As the season progresses, however, the aspect of the vegetation changes and by midsummer there are many typical prairie species. Most of these are apparently survivors of the previously widespread prairie of the central states. Several are western species, some of which are at the eastern borders of their range; others are probably extraneous.

UPLAND FOREST.—The transect method was used in making a quantitative study of the woody plants in the various plant communities. Each quadrat was 50 meters in length and 10 meters in width, and fifty quadrats were studied in each plant community. A record was made only of trees 6 inches or more in diameter, breast high, and of shrubs and small trees 6 feet or more in height. The data in the tables list the species and give: (1) the number of quadrats, in the 50 quadrats, in which each species is found; (2) the average number of individuals in a quadrat, computed on a 50-quadrat basis; and (3) the dominance index. The dominance index was obtained by multiplying the average number of quadrats in which a species was found by the average number of individuals of that species in a quadrat.

In the upland forest (table I) the 50 quadrats were distributed over 20 miles of upland bordering the Illinois River. Most of the

woodlands of the region have been cut over, burned, and grazed; hence their resemblance to the original stand is questioned. From the size and apparent age of the trees, one may conclude that some of them in a few of the timber tracts persist from the virgin stand.

TABLE I

QUANTITATIVE EVALUATION OF TREES AND SHRUBS OF
GREATEST FREQUENCE IN UPLAND FOREST

SPECIES	NO. QUADRATS CONTAINING SPECIES	AVERAGE NO. INDIVIDUALS PER QUADRAT	DOMINANCE INDEX
Trees			
Quercus alba............	50	10.0	500
Q. velutina.......	50	4.2	210
Q. rubra.............. ..	50	3.5	175
Carya alba......	47	3.3	155
C. ovata...............	38	4.0	152
Acer saccharum	20	2.7	54
Fraxinus americana.......	23	1.8	41
Acer rubrum........... ..	14	2.3	32
Juglans nigra........ .. .	19	1.6	30
Quercus muhlenbergii .. .	5	1.6	8
Morus rubra......	5	1.0	5
Smaller trees and shrubs*			
Cercis canadensis........	16	4.0	64
Cornus florida....... ...	25	2.5	61
Sassafras variifolium	11	4.0	44
Rhus glabra........ .	3	8.0	24
Viburnum rufidulum	7	3.0	21
Cornus asperifolia.. . ..	8	1.0	8

* *Ceanothus americanus* is the most common and widely distributed of the shrubs but was not recorded because of its small size.

The tree species as recorded by the dominance index, together with the list of woody and herbaceous plants, indicate that the upland forest is a typical oak-hickory forest association.

TREES OF INFREQUENT OCCURRENCE

Diospyros virginiana   Ulmus americana   .
Aesculus glabra   U. fulva
Prunus serotina   Juglans cinerea
P. americana   Quercus macrocarpa
Juniperus virginiana

SMALL TREES AND SHRUBS OF INFREQUENT OCCURRENCE

Rhus canadensis
Rubus recurvans
R. villosus
Hydrangea arborescens
Ribes gracile
Crataegus spp.
Evonymus atropurpureus
Amelanchier canadensis

Pyrus ioensis
Zanthoxylum americanum
Rosa setigera
Viburnum prunifolium
Ptelea trifoliata
Staphylea trifolia
Rhamnus lanceolata

VINES

Rhus toxicodendron
Psedera quinquefolia
Smilax rotundifolia
S. hispida

Celastrus scandens
Vitis aestivalis
Tecoma radicans

HERBS

Spring

Class 1

Claytonia virginica
Phlox divaricata
Podophyllum peltatum
Galium spp.
Phlox pilosa
Dicentra cucullaria

Ranunculus hispidus
Trillium recurvatum
Dicentra canadensis
Viola sororia
Erythronium albidum

Class 2

Asarum canadense
Triosteum aurantiacum
Antennaria plantaginifolia
Erigeron pulchellus
Potentilla canadensis
Monarda bradburiana
Sanicula gregaria

Uvularia grandiflora
Comandra umbellata
Hepatica triloba
Camassia esculenta
Nothoscordum bivalve
Uvularia perfoliata

Class 3

Lithospermum canescens	Oxalis violacea
Hybanthus concolor	Pentstemon gracilis
Oxalis stricta	Polygonatum commutatum
Asclepias quadrifolia	Physalis subglabrata
Sphenopholis obtusata	Scutellaria versicolor
Brauneria pallida	Allium canadense
Apocynum cannabinum	Castilleja coccinea
var. pubescens	Dodecatheon meadia
Poa pratensis	Actaea rubra
Actaea alba	Viola rafinesquii

Summer

Class 1

Amorpha canescens	Euphorbia corollata
Galium circaezans	Cuscuta coryli
G. pilosum	Gillenia stipulata
Cuscuta cuspidata	

Class 2

Monarda fistulosa	Physalis pubescens
M. punctata	Psoralea onobrychis
Gerardia grandiflora	Anemone virginiana
Pycnanthemum flexuosum	Petalostemum purpureum

Class 3

Asclepias purpurascens	Geum canadense
Oxalis corniculata	Hystrix patula
Asclepias verticillata	Eupatorium urticaefolium
Petalostemum candidum	Eryngium yuccifolium
Ruellia ciliosa	Lilium philadelphicum

Fall

Class 1

Eupatorium torreyanum	Solidago spp.
Desmodium sessilifolium	Helianthus hirsutus

Class 2

Gerardia tenuifolia	Baptisia leucantha
Vernonia altissima	Aster spp.

Class 3

Cassia nictitans	L. squarrosa
Liatris cylindracea	L. scariosa

BLUFF

LIMESTONE BLUFF.—There are two groups of plants commonly found in the limestone bluff habitat, those growing upon the bare rock or in crevices containing little or no soil and those living in crevices or depressions having considerable soil. All divisions of the plant kingdom occurred among the first group of plants. The lichens and mosses were unidentified. Two genera of ferns were named, *Pellaea atropurpurea* and *Cheilanthes feei*. Although usually requiring more soil, *Heuchera americana, Houstonia* sp., and *Elymus canadensis* were the seed plants found in this group.

The second plant group here listed is larger, and it is more variable in its soil requirements than is the first plant group. Among the species listed are those which are also found on the hilltop and in the talus slope forest.

WOODY PLANTS

Class 1

Psedera quinquefolia	Celastrus scandens
Rhus toxicodendron	Vitis aestivalis

Class 2

Hydrangea arborescens	Staphylea trifolia
Lonicera sempervirens	Juniperus virginiana
Rhus canadensis	Cercis canadensis
Ptelea trifoliata	Cornus asperifolia

Class 3

Rhamnus lanceolata	Ceanothus americanus
Viburnum prunifolium	Gymnocladus dioica
Amelanchier canadensis	

HERBS

Spring

Class 1

Aquilegia canadensis   Pellaea atropurpurea
Houstonia lanceolata   Cheilanthes feei
Heuchera americana

Class 2

Triosteum aurantiacum   Phlox pilosa
Comandra umbellata

Class 3

Oxalis violacea   Monarda bradburiana
Dodecatheon meadia   Pentstemon gracilis
Oxalis stricta   Polygonatum commutatum

Summer

Class 1

Houstonia lanceolata

Class 2

Campanula americana

Fall

Class 2

Elymus canadensis   Solidago spp.

The spring flora in this plant community consists of but few species. These, however, constitute the largest number of herbaceous plants. The number of new species and of persisting plants diminishes throughout the summer, and by September the herbaceous vegetation has almost entirely disappeared.

TRANSITION

HILLSIDE-TALUS SLOPE FOREST.—Between the floodplain terraces and the bluffs there are steep slopes upon which grow the hillside-talus slope forest. The quantitative analysis of the woody vegetation of this forest is given in tables II and III.

Owing to the cutting of the red, white, and black oaks for use as timber and firewood, the dominance index of the tree species as given in table II probably does not give a picture of the virgin forest that

TABLE II

QUANTITATIVE EVALUATION OF TREES OF GREATEST FREQUENCE IN HILLSIDE-TALUS SLOPE FOREST

Species	No. quadrats containing species	Average no. individuals per quadrat	Dominance index
Acer saccharum............	50	10.2	510
Quercus muhlenbergii.......	48	7.1	341
Q. rubra..................	36	7.1	266
Tilia americana............	46	3.7	170
Juglans nigra..............	42	3.5	147
Gymnocladus dioica........	27	3.7	100
Carya ovata...............	30	2.5	75
C. alba...................	33	2.0	66
Acer rubrum...............	31	2.1	65
Ulmus americana..........	26	2.4	62
Aesculus glabra............	20	2.3	46
Juglans cinerea............	20	2.1	42
Celtis occidentalis..........	21	2.0	42
Ulmus fulva...............	25	1.5	38
Quercus velutina...........	8	4.0	32
Robinia pseudo-acacia......	9	2.3	21
Fraxinus americana........	17	1.2	20
Carya glabra..............	10	1.4	14

TABLE III

QUANTITATIVE EVALUATION OF SMALL TREES AND SHRUBS OF GREATEST FREQUENCE IN THE HILLSIDE-TALUS SLOPE FOREST

Species *	No. quadrats containing species	Average no. individuals per quadrat	Dominance index
Cercis canadensis...........	37	4.7	174
Staphylea trifolia...........	28	3.8	106
Cornus asperifolia..........	30	3.3	99
Ptelea trifoliata............	21	4.7	99
Asimina triloba............	16	2.8	45
Cornus florida.............	20	2.0	40
Sassafras variifolium........	14	2.0	28
Rhus glabra...............	13	2.0	26
Diospyros virginiana........	8	1.6	13
Carpinus caroliniana........	5	2.0	10
Ostrya virginiana..........	4	2.0	8

* *Hydrangea arborescens* was not recorded because of its small size, although it is one of the most abundant and widespread shrubs in this forest.

occupied this plant community. Lists of the infrequent woody plants and of the herbs in the undergrowth are included in this study. A larger number of woody species, and possibly as many, if not more, herbaceous species are found here than in any other habitat in this region.

<div align="center">TREES OF INFREQUENT OCCURRENCE</div>

Prunus serotina	Carya illinoensis
Quercus alba	C. cordiformis
Morus rubra	Platanus occidentalis
Acer negundo	Gleditsia triacanthos
Quercus macrocarpa	Populus alba
Q. imbricaria	
Fraxinus pennsylvanica	
var. lanceolata	

<div align="center">SMALL TREES AND SHRUBS OF INFREQUENT OCCURRENCE</div>

Rubus spp.	Crataegus spp.
Sambucus canadensis	Benzoin aestivale
Viburnum rufidulum	Ribes gracile
Amelanchier canadensis	Rhamnus lanceolata
Juniperus virginiana	

<div align="center">VINES</div>

Rhus toxicodendron	Vitis vulpina
Psedera quinquefolia	Smilax rotundifolia
Menispermum canadense	Humulus lupulus
Vitis aestivalis	Celastrus scandens
Cissus ampelopsis	Clematis pitcheri

<div align="center">HERBS</div>

Spring

<div align="center">Class 1</div>

Claytonia virginica	Trillium recurvatum
Ranunculus hispidus	Polystichum acrostichoides
Viola sororia	Geranium maculatum
Podophyllum peltatum	Adiantum pedatum
Phlox divaricata	Arisaema triphyllum
Aquilegia canadensis	Delphinium tricorne

Dicentra cucullaria

Galium pilosum

Asarum canadense

Anemonella thalictroides

Onoclea sensibilis

Cystopteris fragilis

Class 2

Smilacina racemosa

Dicentra canadensis

Polygonatum commutatum

Sanguinaria canadensis

Anemone canadensis

Menispermum canadense

Geranium carolinianum

Botrychium virginianum

Osmorhiza claytoni

Sanicula gregaria

Cerastium arvense var. oblongifolium

Uvularia grandiflora

Dentaria laciniata

Triosteum aurantiacum

Caulophyllum thalictroides

Viola pubescens

Aruncus sylvester

Mertensia virginica

Hydrophyllum virginianum

Asplenium acrostichoides

Class 3

Ruellia strepens

Hepatica triloba

Hybanthus concolor

Osmorhiza longistylis

Smilax herbacea

Monarda bradburiana

Panax quinquefolium

Pedicularis canadensis

Trillium grandiflorum

Arabis laevigata

Oxybaphus nyctagineus

Heuchera americana

Cypripedium parviflorum

Sphenopholis obtusata

Liparis liliifolia

Hydrastis canadensis

Actaea rubra

Summer

Class 1

Podophyllum peltatum

Phlox divaricata

Galium spp.

Adiantum pedatum

Aquilegia canadensis

Polystichum acrostichoides

Desmodium bracteosum

Impatiens biflora

I. pallida

Onoclea sensibilis

Cystopteris fragilis

Class 2

Cuscuta coryli
Aralia racemosa
Botrychium virginianum
Campanula americana
Eupatorium purpureum
Cryptotaenia canadensis
Asplenium acrostichoides
Anemone virginiana
Phlox paniculata

Tradescantia reflexa
Dioscorea villosa
Hydrophyllum virginia-
  num
Polymnia canadensis
Aruncus sylvester
Scrophularia marilandica
Phytolacca decandra

Class 3

Eupatorium urticaefolium
Aspidium marginale
Geum canadense
Circaea lutetiana
Lobelia siphilitica
Hystrix patula
Heliopsis scabra
Gerardia flava
Gillenia stipulata
Actinomeris alternifolia

Sicyos angulatus
Lilium canadense
Scutellaria pilosa
Panax quinquefolium
Hydrastis canadensis
Rudbeckia triloba
Cystopteris bulbifera
Polymnia canadensis var.
  radiata

Fall

Class 1

Eupatorium serotinum
Kuhnia eupatorioides

Gnaphalium polycephalum
Desmodium sessilifolium

Class 2

Setaria glauca
Cirsium lanceolatum
Aster spp.

Conobea multifida
Actinomeris alternifolia

Class 3

Eupatorium sessilifolium
Brauneria purpurea
Panicum praecocius
Anychia canadensis
Eupatorium urticaefolium
Cassia nictitans

Digitaria sanguinalis
Cirsium discolor
Panicum clandestinum
Muhlenbergia sobolifera
Brachyelytrum erectum
Eragrostis capillaris

TALUS SLOPE-FLOODPLAIN TRANSITION FOREST.—The floodplain terrace, which lies between the talus slope and the flat bottomland, slopes gently toward the river, and upon it grows the talus slope-floodplain transition forest.

In this transition zone is found a mixture of forest trees (table IV). The American elm, burr oak, pecan, sycamore, hackberry, green ash, honey locust, and soft maple are representative floodplain trees; while walnut, butternut, red bud, white ash, and Kentucky coffee

TABLE IV

QUANTITATIVE EVALUATION OF TREES OF GREATEST FREQUENCE
IN TALUS SLOPE-FLOODPLAIN TRANSITION FOREST

SPECIES	NO. QUADRATS CONTAINING SPECIES	AVERAGE NO. INDIVIDUALS PER QUADRAT	DOMINANCE INDEX
Ulmus americana..........	48	10	480
Platanus occidentalis.......	50	6.5	325
Celtis occidentalis..........	40	5	200
Cercis canadensis..........	33	3	99
Acer saccharinum..........	36	3.2	85
Carya illinoensis...........	27	2.8	76
Acer negundo..............	23	3	69
Quercus macrocarpa........	27	2.3	62
Gleditsia triacanthos..... ..	21	1.8	58
Cornus asperifolia..........	17	3	51
Gymnocladus dioica........	22	2	44
Diospyros virginiana........	18	2	36
Fraxinus americana........	16	2	32
Juglans nigra.....	18	1.3	23
Tilia americana............	12	1.3	16
Juglans cinerea............	8	1.5	12

bean are typical hillside-talus slope trees. Significant is the fact that the American elm, sycamore, burr oak, box elder, and hackberry attain their maximum concentration or dominance in this habitat.

TREES AND SHRUBS OF INFREQUENT OCCURRENCE

Crataegus spp.   Prunus serotina
Carya cordiformis   Asimina triloba
Quercus muhlenbergii   Populus deltoides
Adelia acuminata   Sassafras variifolium
Salix longifolia   Sambucus canadensis
Ulmus fulva

VINES

Psedera quinquefolia   Smilax hispida
Rhus toxicodendron   Tecoma radicans
Vitis vulpina   Menispermum canadense
Smilax rotundifolia   Cissus ampelopsis

HERBS

Spring

Class 1

Claytonia virginica   Ranunculus hispidus
Phlox divaricata   Smilacina racemosa
Podophyllum peltatum   Hydrophyllum virginia-
Ranunculus septentrionalis   num
Delphinium tricorne   Arisaema dracontium
Trillium recurvatum   Amsonia tabernaemontana
Ranunculus abortivus   Potentilla monspeliensis
Capsella bursa-pastoris   Veronica officinalis
Cerastium arvense var. ob-   Arabis virginica
  longifolium   Veronica peregrina
Mertensia virginica   Chaerophyllum procum-
Anemonella thalictroides   bens
Viola sororia   Rumex acetosella
Anemone canadensis   Viola cucullata
Arisaema triphyllum   Osmorhiza claytoni
Geranium carolinianum   O. longistylis

Class 2

Solanum triflorum   Senecio integerrimus
Cerastium vulgatum   Botrychium virginianum
Specularia perfoliata   Apocynum cannabinum
Convolvulus sepium   var. pubescens
Viola rafinesquii   Ruellia strepens
Oxybaphus nyctagineus

Class 3

Lythrum alatum   Plantago lanceolata
Smilax herbacea   Pedicularis canadensis

Bromus secalinus
Plantago virginica
Marrubium vulgare
Achillea millefolium

Malva rotundifolia
Arabis patens
Amorpha fruticosa
Festuca elatior

Summer

Class 1

Sicyos angulatus
Campanula americana
Tradescantia pilosa

Cassia chamaecrista var.
   robusta

Class 2

Impatiens biflora
Ruellia ciliosa
Desmodium bracteosum
Clematis pitcheri

Veronica virginica
Eupatorium purpureum
Phytolacca decandra

Class 3

Prunella vulgaris
Silene stellata
Gerardia flava
Leersia virginica
Cacalia reniformis
Aspidium marginale
Polygonum setaceum
Silphium perfoliatum

Saponaria officinalis
Rudbeckia laciniata
Lobelia inflata
Anemone virginiana
Hystrix patula
Verbascum thapsus
Nepeta cataria

Fall

Class 1

Eupatorium serotinum
Solidago serotina

Heliopsis scabra
Chenopodium glaucum

Class 2

Eupatorium perfoliatum
Rudbeckia triloba

Humulus lupulus
Cuscuta glomerata

Class 3

Eupatorium urticaefolium
Conobea multifida

Panicum clandestinum
Muhlenbergia sobolifera

Brachyelytrum erectum   Croton glandulosus var.
Euphorbia dentata   septentrionalis
Sorghum halapense

FLOODPLAIN

The floodplain or bottomland includes the remaining lowland vegetation between the terrace and the channel of the Illinois River. This topographic unit has provided the best opportunity for the study of the hydrarch plant succession, which may be seen in forest, prairie, lake, river bank, and island plant communities.

TABLE V

QUANTITATIVE EVALUATION OF TREES OF GREATEST
FREQUENCE IN THE FLOODPLAIN FOREST

SPECIES	NO. QUADRATS CONTAINING SPECIES	AVERAGE NO. INDIVIDUALS PER QUADRAT	DOMINANCE INDEX
Acer saccharinum..........	48	8.7	418
Ulmus americana..........	50	6.4	320
Carya illinoensis..........	47	4.1	193
Quercus palustris..........	45	3 4	153
Adelia acuminata..........	36	3.7	133
Crataegus sp........,........	36	3.2	115
Celtis occidentalis..........	38	3.0	114
Fraxinus pennsylvanica var. lanceolata...............	42	2.6	109
Betula nigra.........	23	3.0	69
Gleditsia triacanthos.......	16	2.0	32
Diospyros virginiana........	16	1.6	26
Populus deltoides..........	12	2.0	24

FLOODPLAIN FOREST.—The habitat of the floodplain forest varied from the moister area bordering swamps, sloughs, or river bank to the higher, drier parts of the floodplain.

The dominance index of the floodplain forest (table V) shows that the soft maple and American elm are the dominant trees; pecan, hackberry, pin oak, swamp privet, and *Crataegus* are second in abundance; while river birch, honey locust, persimmon, and cottonwood occur in lesser abundance. The cottonwood, occurring most abundantly in moist or pioneer areas, was the pioneer tree of the floodplain forest. It persisted in the early stages of floodplain succession but was rarely found in areas where the climax or subclimax forest was established.

The pecan and pin oak were occasionally important, forming pure stands locally. The cutting of less desirable trees may account for this dominance of the pecan. In a few limited areas, north of the region studied, the pin oak occupied 40 per cent of the timber stand.

In early spring in the lower, wetter parts of the floodplain forest occur *Senecio glabellus* and *Radicula palustris*, and colonies of *Amsonia tabernaemontana*, *Erigeron*, and *Apocynum cannabinum*. The higher, better drained parts of this forest contain several species of *Ranunculus* (*R. septentrionalis* being the most common), *Phlox divaricata*, *Anemone* spp., *Podophyllum*, *Claytonia*, *Trillium*, *Geranium*, and *Viola* spp. Summer finds an abundance of *Polygonum*, *Steironema*, *Stachys*, *Chenopodium*, *Lysimachia*, and *Teucrium*. Species of these genera may be intermingled, or any one of them may form societies of varied extent. In late summer and fall composites dominate the habitat, and *Eupatorium serotinum* is the most abundant species. *Vernonia illinoensis* and *V. missurica* are conspicuous and important, and commonly form extensive societies in the more open lowland forest. In damp, well shaded parts of the forest, *Laportea* is frequently dominant, and associated with it as a subdominant may be *Impatiens biflora*. In September *Boltonia asteroides* is common in the more open areas, *Vernonia* spp. persist, *Matricaria suaveolens* appears, and *Physostegia virginiana* occurs along the forest edge. Species of *Solidago* and *Bidens* occur sparingly in the floodplain forest but are abundant in the adjacent floodplain prairie and abandoned fields.

<div align="center">

TREES OF INFREQUENT OCCURRENCE

</div>

Platanus occidentalis	Ulmus fulva
Carya laciniosa	Gleditsia aquatica
Morus rubra	Quercus lyrata
Quercus bicolor·	

<div align="center">

VINES

</div>

Smilax rotundifolia	Psedera quinquefolia
S. hispida	Rhus toxicodendron
Menispermum canadense	Tecoma radicans
Vitis vulpina	Cissus ampelopsis
V. cordifolia	

Spring

Class 1

Radicula palustris
Senecio glabellus
Phlox divaricata
Ranunculus septentrionalis
Potentilla monspeliensis
Claytonia virginica
Podophyllum peltatum

Arisaema triphyllum
Geranium maculatum
Anemone canadensis
Trillium recurvatum
Hydrophyllum virginia-
num
Arisaema dracontium

Class 2

Ranunculus abortivus
Chaerophyllum procum-
bens
Erigeron pulchellus
E. philadelphicus
Galium triflorum

Apocynum cannabinum
var. pubescens
Amsonia tabernaemontana
Viola cucullata
V. pubescens
V. missouriensis

Class 3

Spermacoce glabra
Iris versicolor
Ranunculus sceleratus

Ranunculus micranthus
Dicentra canadensis

Summer

Class 1

Lysimachia quadrifolia
Chenopodium album
Impatiens biflora

Stachys tenuifolia
Steironema ciliatum
Teucrium canadense

Class 2

Ammannia coccinea
Amsonia tabernaemontana
Verbena urticaefolia
Eupatorium coelestinum
Polygonum virginianum
Oenothera muricata var.
canescens

O. biennis
Galium triflorum
Polygonum pennsylvani-
cum
Phytolacca decandra

Class 3

Clematis pitcheri   Scutellaria lateriflora
Polygonum longistylum   Anthemis cotula
Solanum carolinense   Mentha piperita
Saururus cernuus

Fall

Class 1

Eupatorium serotinum   Vernonia missurica
Laportea canadensis   Boltonia asteroides
Vernonia illinoensis

Class 2

Solidago spp.   Boltonia asteroides var. de-
Cuscuta glomerata   currens
Helenium autumnale   Physostegia virginiana
Boehmeria cylindrica

Class 3

Solanum nigrum   Aster paniculatus
Matricaria suaveolens   Lobelia cardinalis

FLOODPLAIN PRAIRIE OR SAVANNA.—Unforested areas covered with prairie vegetation were commonly found on the floodplain. A zone of floodplain forest, from one-half to one mile in width, bordered the river channel and surrounded the lakes, whereas the prairie associations occurred on the somewhat higher ground between the river and the bluffs. *Spartina michauxiana* and *Panicum virgatum* constituted the dominant associations of this community.

Prairie areas on a floodplain were included in the investigations by BRENDEL (3) in the Illinois River valley near Peoria, by SAMPSON (11) along the Mississippi River near Savanna, and by CLEMENTS and WEAVER (4) along the western tributaries of the Mississippi River. GLEASON (5) has discussed the relationship of forest and prairie. The writer has discussed the prairie in detail (13).

FLOODPLAIN LAKES.—The lakes of the floodplain are classified as permanent and temporary forest lakes, and prairie lakes. The flood-

plain forest lakes are usually found in the forest near the river channel. The floodplain prairie lakes, lying in the wet prairie, are more frequently farther from the river channel, on higher ground, and shallower than the forest lakes. Each of these types of lakes has a flora which distinguishes it. Here the hydrarch succession from water through slough or swamp to the forest or to the prairie was easily determined.

PERMANENT FOREST LAKE.—Three definite zones of woody plants were observed between the floodplain forest and the permanent forest lake. The first zone retains honey locust and green ash from the floodplain forest. These occur in equal abundance with swamp privet (*Adelia acuminata*) and *Salix nigra*. With these are found also *S. fragilis*, *S. alba*, *S. longifolia*, and *S. amygdaloides*. Rarely does this zone escape submergence to a foot or more for several weeks each spring. The second zone is comprised of *Adelia acuminata* and button bush (*Cephalanthus occidentalis*), together with a few green ash and honey locust trees. Occasionally swamp privet dominates the zone, forming dense, almost impenetrable thickets. This zone is submerged in a foot or more of water from two to six months each year. The third zone consists of an irregular, more or less discontinuous belt of *Cephalanthus*. Except in extremely dry years, this zone is submerged to a depth of a few inches to three or more feet of water during the entire year.

The flora within and surrounding the floodplain lakes of the middle Mississippi River valley (6) and the upper Illinois River valley (3) is richer in species than is that of the floodplain lakes in the lower Illinois River valley. This may be accounted for by the fact that the condition of the habitat of the lakes in the lower Illinois valley is rendered unstable because of periodic floods which sweep the vegetation from these lakes.

The herbaceous plants occurring within and around the lakes of the lower valley have been separated into three groups: the aquatic plants, which include the floating and submerged plants; the amphibious plants, which comprise floating leaves, submerged, and emerged aquatic plants at the water's edge; and emerged aquatic and swamp plants. The species collected are given in the following list.

HERBS

Aquatic plants

Class 1

Lemna spp.   Spirodela polyrhyza
Wolffia punctata

Class 2

Ceratophyllum demersum   Potamogeton spp.
Elodea canadensis

Class 3

Nelumbo lutea   Pontederia cordata

Amphibious plants

Class 1

Polygonum pennsylvani-   Rumex verticillatus
  cum   R. crispus
P. longistylum

Class 2

Alisma plantago-aquatica   Radicula nasturtium-aqua-
Saururus cernuus   ticum
Ludvigia palustris   Carex spp.

Class 3

Polygonum aquaticum   Cicuta maculata
Sium cicutaefolium   Sagittaria latifolia
Radicula aquatica   S. graminea

Emerged aquatics and swamp plants

Class 1

Radicula palustris   Lippia lanceolata

Class 2

Myosurus minimus   Boehmeria cylindrica
Arabis virginica

Class 3

Ranunculus micranthus	Ranunculus sceleratus
Thlaspi arvense	Iris versicolor
Spermacoce glabra	Equisetum arvense
Bidens bipinnata	Polygonum virginianum
Samolus floribundus	

TEMPORARY FOREST LAKES.—An opportunity is afforded in these shallow temporary forest lakes to observe a more advanced stage in hydrarch succession. What was at one time the outermost part of the lake bed is now occupied by floodplain forest trees. In a zone between these trees and the lake are growing ash, locust, maple, pin oak, and pecan trees. The center of some of these lakes may be occupied by swamp privet, willows, or button bush. These trees and shrubs impede the passage of flood water, and the obstruction causes the deposition of silt. The habitat becomes drier and thereby accelerates the development of the succession to the low-valley climax or floodplain forest.

While three groups of herbaceous plants were found, there were but few species in each group. Three genera of floating aquatic plants were seen, *Lemna*, *Wolffia*, and *Spirodela*. The amphibious plants commonly found were *Saururus*, *Polygonum*, *Jussiaea*, *Radicula*, *Rumex*, and *Sagittaria*. Since these lakes become dry in late summer, the fall aspect of the former water's edge and lake bed is different from that of the permanent forest lake. This space is now occupied by such weed genera as *Chenopodium*, *Bidens*, *Arabis*, *Thalaspi*, and *Rumex*.

FLOODPLAIN PRAIRIE LAKE.—The only woody species found on the wet prairie are an occasional willow tree or clumps of shrubs of willow or button bush. The numbers of herbaceous species of aquatic and amphibious plants of the floodplain prairie lake are about the same, but are more abundant than those of the forest lakes. *Saururus* does not grow here. The following list of herbaceous plants shows that the number of species of submerged and swamp plants of the wet prairie immediately surrounding these lakes is more than twice that of the temporary forest lakes.

Emerged aquatic and swamp plants

Class 1

Eleocharis palustris	Chenopodium spp.
Iva ciliata	Eragrostis hypnoides
Xanthium canadense	Panicum virgatum
Juncus effusus	Lippia lanceolata
Rumex crispus	Spartina michauxiana
Alopecurus geniculatus	Bromus secalinus
Amaranthus spp.	Bidens cernua
Carex spp.	Scirpus fluviatilis
Echinochloa crusgalli	Sparganium eurycarpum

Class 2

Scirpus georgianus	Hibiscus militaris
Mimulus alatus	Ambrosia trifida
Alisma plantago-aquatica	Bidens comosa
Sagittaria latifolia	Eclipta alba
Cyperus spp.	

Class 3

Mimulus ringens	Vernonia fasciculata
Cicuta maculata	Ammannia coccinea
Steironema ciliatum	Portulaca oleracea
Eupatorium coelestinum	Penthorum sedoides

Eleocharis palustris is almost always present in this habitat, where it frequently either dominates the muddy shore line or forms dense mats in semi-dry lake beds. *Typha latifolia* occasionally forms rather extensive colonies, *Scirpus fluviatilis* completely fills a few shallow lakes, but it does not seem to have a wide distribution. *Nelumbo lutea* probably once had a wide distribution in the floodplain, judging from the number of seeds and floral receptacles found in dry lake beds. At present it is confined to a few small colonies in drainage sloughs and lake remnants.

Ponds and sloughs have fewer plant species than the larger lakes and are frequently dominated by a single species. They are often

occupied exclusively by *Typha, Sagittaria, Alisma, Polygonum, Sparganium, Hibiscus*, or *Rumex*.

RIVER BANK.—The plant succession of the river bank or shore line which lies between the water's edge and the floodplain forest may be seen in four zones. The study of the lower zones of this succession is simple, since the fluctuating water level effectively prevents the development of a widely diversified group of life forms.

Within the first zone, which is near the water's edge, seedlings of *Salix, Acer*, and *Populus* develop. Most of these seedlings eventually will be destroyed because the water level in most of these habitats is only relatively stable. Herbs growing in this zone are *Polygonum* and *Rumex, Eragrostis hypnoides, Lippia lanceolata, Juncus effusus, Eleocharis acicularis*, and *Xanthium canadense*. Less common herbs are *Jussiaea diffusa, Alisma plantago-aquatica, Sagittaria latifolia, Spermacoce glabra, Penthorum sedoides*, and *Eclipta alba*. In the second zone are found larger tree seedlings, or sprouts. This zone usually consists mostly of a somewhat dense stand of willows. There occurs in the third zone a series of belts of willow, each belt increasing in age and height as the distance from the water's edge becomes greater. Included in this zone also are a few swamp privets, cottonwoods, and maples. The pioneer trees of the river bank succession are the willow and the cottonwood. The fourth zone, which joins the floodplain forest, is the most stabilized. It is composed of mature willows, cottonwoods, hawthorns, and medium aged maples. *Vitis vulpina*, and to a lesser extent *V. palmata* and *V. cordifolia*, often form dense mats over the willow fringe. The trees that grow along the shore in the order in which they most commonly occur are *Salix nigra, S. fragilis, S. amygdaloides, S. discolor, Populus deltoides, Acer saccharinum, Adelia acuminata, Salix alba. Crataegus* sp., *Salix longifolia*, and *Platanus occidentalis*.

The following herbs were observed on the more stabilized zones of the river bank:

HERBS

Spring

Class 1

| Radicula palustris | Myosurus minimus |
| Senecio glabellus | Equisetum arvense |

Class 2

Arabis virginica

Sisymbrium canescens

Thlaspi arvense

Lepidium virginicum

Class 3

Ranunculus abortivus

Potentilla monspeliensis

Chaerophyllum procumbens

Summer

Class 1

Polygonum pennsylvanicum

Chenopodium album

Lippia lanceolata

Polygonum lapathifolium

Rumex crispus

Chenopodium glaucum

Convolvulus sepium

Class 2

Spermacoce glabra

Oenothera muricata

Gonolobus laevis

Polygonum longistylum

P. aviculare

Mimulus alatus

Class 3

Alisma plantago-aquatica

Jussiaea diffusa

Samolus floribundus

Baptisia leucantha

Hibiscus militaris

Fall

Class 1

Amaranthus retroflexus

A. blitoides

Carex spp.

Eleocharis acicularis

Xanthium canadense

Eupatorium serotinum

Eragrostis hypnoides

Bidens comosa

Juncus effusus

Class 2

Acnida tuberculata

Amaranthus graecizans

Portulaca oleracea

Polygonum amphibium

Bidens bipinnata

Penthorum sedoides

Bidens frondosa

Commelina communis

Echinochloa crusgalli

Sagittaria latifolia

Eclipta alba

Class 3

Bromus secalinus	Gratiola virginiana
Cuscuta compacta	Mimulus ringens
Bidens cernua	Eleusine indica
Polygonum hydropiper-oides	Radicula armoracia

Both primary and secondary successions are represented along the river bank. Primary succession is observed below the mouth of entering tributaries where the bank is encroaching upon the river. Secondary succession is constantly taking place in the zones nearest the water's edge because of the almost annual destruction of the river bank plants. Ice jams along the river contribute largely to this destruction.

ISLANDS.—The flora of the islands is separated into two groups, that of the small and that of the large islands. The small islands have a maximum length of 800 yards, while the large islands vary from 800 yards to 3 or 4 miles in length, and may have a maximum width of 1 mile.

The small islands are characterized by a pioneer type of vegetation, and show the earliest stages in the hydrarch succession; the larger islands include a more advanced stage in this succession. The islands are generally from 1 to 3 feet lower than the floodplain of the mainland. This fact may explain why the succession on the largest islands does not reach the final stage of the climax floodplain forest.

The farming that was attempted on several of these islands met with the same or even less success than that on the unleveed floodplain. Abandoned farm areas showed present stages of plant growth in which the plant species corresponded with those of the primary plant succession of the islands.

SMALL ISLANDS.—The small islands are irregularly oval in shape, rounded at the upper end and tapering toward the downstream end. Since the upper end of an island is the oldest and wears away while the lower end builds up, zonation and well defined steps of plant succession were easily recognized.

Total tree counts were made in ten small islands and the results are recorded in figure 1. The lower tip of these islands has the

pioneer stage or first zone (*A*) of the plant succession. This first zone, which is identical with those found at the water's edge on the mainland, forms the outermost plant fringe of the entire island.

Zone E   Percentage
  Maple.................. 45
  Elm.................... 43
  Cottonwood............ 8
  Sycamore.............. 4

Zone D
  Maple.................. 45
  Cottonwood............ 20
  Willow................. 15
  Elm.................... 15
  Swamp privet.......... 5

Zone C
  Willow................. 70
  Maple.................. 15
  Cottonwood............ 10
  Swamp privet.......... 5

Zone B
  Willow................. 90
  Swamp privet.......... 10

Zone A
  Herbs, willow sprouts
    and seedlings
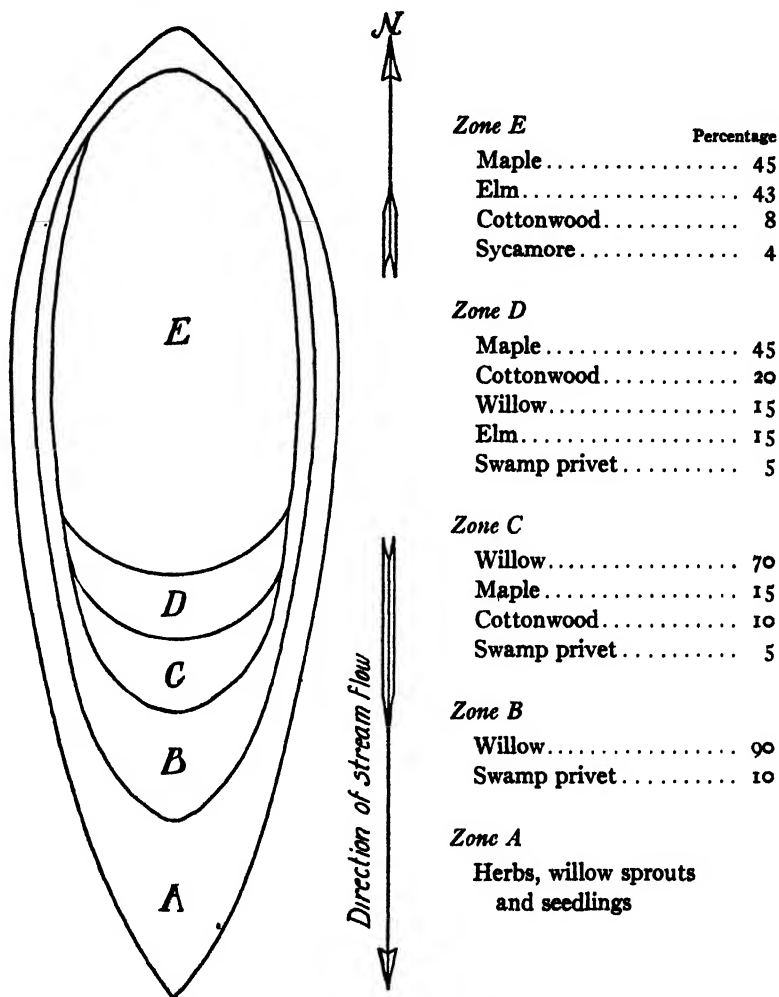
FIG. 1.—Floral analysis of small islands in lower Illinois River valley

Farther up from the lower end of the island occurs the second zone (*B*), which is a consociation of young willows with a few swamp privets and cottonwood sprouts. In the third zone (*C*) the per-

centage of willows decreases, cottonwoods become more important, and young maples appear. The fourth zone (*D*) exhibits two features, the occurrence of elms and the peak of cottonwood concentration. Willows decrease abruptly in this zone. Maples are found in approximately the same proportion on the upper part of the islands. In the fifth zone (*E*) the upper and interior three-fifths of the island has attained a condition of relative stability. Soft maples and elms are dominant, with the former slightly more numerous; cottonwoods decrease to 6–10 per cent of the stand; sycamores occur occasionally. The herbaceous cover differs little from that of the lower floodplain forest of the mainland.

LARGE ISLANDS.—At their lower end, the large islands exhibit the same general type of zonation as the small islands. The forest on the upper end differs primarily from that of the small islands in having an increased number of species and in having attained a state of relative stability. This most stable zone may be designated as the sixth zone or climax island forest. Although essentially the same species occur here as in the climax floodplain forest, the data in table VI show that the dominance index of some of the species in the two habitats varies.

The first five tree species listed in table VI are the most abundant in both the sixth zone or island climax forest and the climax floodplain forest of the mainland. The dominance index for these island species is somewhat less for the island than for the mainland species, except for the elm, which becomes the dominant tree. On the other hand, among the subdominant species the cottonwood, honey locust, and the sycamore attain a much higher dominance index in this habitat than on the mainland. Apparently the elm and the three species just given are more water tolerant and have a wider adaptation to soils containing less humus than have the other species. The hackberry, ash, hawthorn, river birch, swamp privet, and persimmon are subdominants which are more widespread and occur in greater numbers in the climax floodplain forest.

The herbaceous plants of the large islands are the same species as those found in the upper part of the small islands and in the low floodplain forest of the mainland.

LEVEES.—Plant succession on artificial levees in the valley has been discussed elsewhere (12).

QUANTITATIVE EVALUATION OF TREES OF GREATEST FREQUENCE ON LARGER
ISLANDS, AND COMPARISON OF DOMINANCE INDEX
WITH FLOODPLAIN FOREST

SPECIES	NO. QUADRATS CONTAINING SPECIES	AVERAGE NO. INDIVIDUALS PER QUADRAT	DOMINANCE INDEX	
			ISLAND FOREST	FLOODPLAIN FOREST
Ulmus americana............	50	7.3	365	320
Acer saccharinum...	50	7.2	360	418
Carya illinoensis............	46	3.7	170	193
Adelia acuminata	37	3.0	111	133
Quercus palustris............	25	4.0	100	153
Populus deltoides........ ..	34	2.9	90	24
Celtis occidentalis.	41	2.1	86	114
Gleditsia triacanthos...	26	2.0	52	32
Platanus occidentalis.........	31	1.5	47	Rare
Fraxinus pennsylvanica var. lanceolata.................	12	2.0	24	109
Crataegus sp............. ..	23	1.0	23	115
Betula nigra.............. ..	13	1.0	13	69
Diospyros virginiana.........	12	1.0	12	26

TREES OF INFREQUENT OCCURRENCE

Carya laciniosa   Acer negundo
Morus rubra

Summary

1. Soil types found in Pike and Calhoun counties at the lower end of the Illinois River were correlated as far as possible with the plant communities growing upon them. The soils in the entire area studied have rather a wide variety of texture and type. There was a small range in the hydrogen-ion concentration of the soils. The variation of the pH of the upland soils was from 6 to 6.6; the floodplain soils was from 7 to 7.8. The pH of the talus slope was 8. The narrow range of pH seems to indicate that it is not an important factor in limiting the distribution of species.

2. A transect of the lower valley affords four general divisions: upland, bluff, transition region, and floodplain. Subdivision of these

results in ten major plant communities: upland pasture and old fields, upland forest, limestone bluff, hillside-talus slope forest, talus slope-floodplain transition forest, floodplain forest, floodplain prairie, floodplain lakes and sloughs, river bank, and islands.

3. The dominance index, derived from a quantitative study of trees 6 inches in diameter breast high and of shrubs over 6 feet in height, was used to determine the types of forest in this region. Herbs and some of the woody plants under 6 feet in height were studied, in the various habitats, in their spring, summer, and fall aspects.

4. The fragments of the original upland prairie that persist between the present areas of pastures and old fields contain few of the prairie plants that were formerly abundant. However, this upland habitat is second richest of all within the range in number of species when introduced ruderals are recorded.

5. The upland forest is a typical midwestern oak-hickory forest. Of all the plant communities, this upland forest ranks fourth in total number of species.

6. The limestone bluff has the smallest number of species of any of the plant communities studied and nearly all of these are herbs which appear in the spring.

7. The hillside-talus slope forest has as its dominant species the hard maple, the chestnut oak and the red oak. This plant community is the richest of all in both herbaceous and woody species. The explanation for this lies perhaps partly in the fact that it has been least disturbed by man, and partly because it is a favorable habitat for a great number of mesophytic plants indigenous to the central states.

8. The talus slope-floodplain transition forest lies above the zone of destructive floods. Cultivation and grazing have brought about the destruction of many of the native species. It contains species from both the hillsides and the floodplain. The elm, the sycamore, and the hackberry are the dominant trees.

9. The floodplain forest has more trees that have a dominance index of 100 or more than has any other forest studied. Soft maple and elm are the dominant trees. The small number of woody plants in the floodplain forest compared with other forests may be accounted

for by the scarcity of shrubs. Woody plants comprise 26 per cent of the total plants recorded for the floodplain forest, while hillside-talus slope forest and talus slope-floodplain forest have 33 per cent. The destructive action of floods doubtless accounts for the small number of perennial herbs and shrubs in this forest.

10. The floodplain prairie or savanna occurs on the broader floodplain areas of the Illinois River, generally a few feet higher than the floodplain forest; hence it is drier and less subject to flooding.

11. The lakes and sloughs have been greatly reduced in number and size by drainage. Both the permanent and temporary floodplain forest lakes and the floodplain prairie lakes show the usual zonation of plants in the hydrarch succession from the water to the forest or to the prairie climax. In general, the number of plant species is much smaller than in many lakes elsewhere, possibly because of the annual destruction of the plants by floods.

12. Four zones of plants constituted the hydrarch succession which occupies the river bank between the water's edge and the floodplain forest. Willow and cottonwood were the pioneer trees. In the third zone the willow occurred in several belts, each of which increased in age and height as the distance from the water's edge became greater.

13. The small islands have a zonation which is similar to that of the river bank. Except at the center of the islands, the habitat is relatively unstable. A quantitative study of the trees in each zone showed that there was a gradual change in tree species, and in the frequency of their occurrence from the less stable zone at the water's edge to the more stable zone at the center of the islands.

14. The lower end of the large islands has a zonation similar to that of the small islands. At the upper end the habitat is more stable, and there is a greater number of plant species than in this position on the smaller islands. A comparison of the dominance index of the trees in the climax island forest and the climax floodplain forest of the mainland indicates that the former has not reached so high a state of development as the climax forest of the mainland.

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LITERATURE CITED

1. ALVORD, J. W., and BURDICK, C. B., The Illinois River and its bottomlands with reference to the conservation of agriculture and fisheries and the control of floods. Report of Illinois River and Lakes Commission. Illinois State Journal Co., State Printers, Springfield, Ill. 1915.

2. BARROWS, H. H., Geography of the middle Illinois valley. Illinois State Geol. Surv. Bull. 15. Urbana, Ill. 1910.

3. BRENDEL, FREDERICK, Flora Peoriana. J. W. Franks & Sons, Peoria, Illinois. 1887.

4. CLEMENTS, F. E., and WEAVER, J. E., Experimental vegetation; the relation of climaxes to climates. Carnegie Inst. Washington. Publ. 355. 1924.

5. GLEASON, H. A., The vegetational history of the middle west. Ann. Assoc. Amer. Geog. 12:39–86. 1922.

6. HUS, HENRI, An ecological cross section of the Mississippi River in the region of St. Louis, Missouri. Report Missouri Bot. Gard. 19:127–258. 1908.

7. Illinois Agric. Expt. Sta., Pike County soils. Soil Report no. 11. Urbana, Ill. 1915.

8. ———, Calhoun County soils. Soil Report no. 53. Urbana, Ill. 1932.

9. Illinois State Geol. Surv., Geologic map of Illinois. Urbana, Ill. 1917.

10. PICKELS, G. W., and LEONARD, F. B., Engineering and legal aspects of land-drainage in Illinois. Illinois State Geol. Surv. Bull. 42. rev. ed. 1929.

11. SAMPSON, H. C., An ecological survey of the prairie vegetation of Illinois. Bull. Illinois State Nat. Hist. Surv. 13:523–577. 1918–1921.

12. TURNER, L. M., Plant succession on levees in the Illinois River valley. Trans. Illinois State Acad. Sci. 24:94–102. 1931.

13. ———, Grassland in the floodplain of the Illinois River. Amer. Mid. Nat. 15:770–780. 1934.

14. United States Weather Bureau, Summaries of climatological data by sections. Bull. W. Reprint of Section no. 57. Central Illinois. 1930.

15. United States Division of Waterways and Engineering Department. Unpublished data taken by permission from records of keeper of dams and locks. Kampsville, Illinois.

HYDROTROPIC RESPONSES OF
ROOTS IN SOIL

W. E. LOOMIS AND L. M. EWAN

(WITH FOUR FIGURES)

A positive response of roots to moisture stimuli is very commonly assumed. The literature shows that this assumption is based largely upon the observations of SACHS (5) and MOLISCH (4), who showed that roots growing through the screen bottom of a moss-filled basket turned at an angle of 45° from the vertical and followed the moist surface of the bottom of the basket; and upon the researches of HOOKER (3), who obtained positive responses of roots to moisture when they were grown in a water vapor gradient and out of direct contact with a moist surface.

SACHS attempted to explain this bending by assuming that the exposed surface of the roots lost more water by evaporation and was therefore cooled below the inner surface, which was in contact with the moist surface. He attempted, with negative results, to obtain a bending away from the moist surface by warming the roots with direct radiation. MOLISCH assumed also that the evaporation on the exposed side of the roots was responsible for the stimulus but he did not attempt to introduce temperature factors. All who have attempted the SACHS experiment know that the "hydrotropic response" of the roots under these conditions depends upon a moderate moisture deficit in the air. As the humidity of the air surrounding the roots approaches 100 they grow downward and show no tendency to follow the moist screen.

In some preliminary experiments, the writers found that when roots were grown in a saturated atmosphere and showed no tendency to follow a moist surface, the temperature of the moist surface was the same as that of the air. When the humidity was decreased to 70 or 80 per cent, the roots of corn followed a moist filter paper at an angle of 45° and the temperature of the filter paper was cooled by evaporation to 5° below air temperature. These results suggest that

the bending of roots in air is associated with temperature differences. The side of the root in contact with a cool moist surface may grow more slowly because of its lower temperature and the unequal growth rate on the two sides of the root cause it to bend toward the moist surface against the effect of geotropism. In HOOKER's (3) experiments the roots were hung vertically in front of a moist blotting paper at distances as great as 10 cm. from the paper. Positive responses were shown at moderate moisture vapor gradients and negative responses at higher gradients. The positive responses were called hydrotropism and the negative responses were assigned to greater drying and less turgor on the side of the root away from the moist surface, an effect which became dominant as the moisture gradient was increased. Temperature differences were not measured but were undoubtedly present.

The hydrotropic responses of roots in air are of considerable academic interest, but they appear to have little relationship to the development of plants under normal conditions. The minimum soil moisture to which plant roots are normally exposed is represented by the wilting percentage or the wilting coefficient of the soil. The wilting percentage is considerably (47 per cent) above the hygroscopic percentage, at which point the soil moisture is stated to be in equilibrium with air saturated with water vapor. It would not be expected, therefore: (1) that vapor gradients would normally be present in soil regions where plant roots were growing; (2) that evaporation rates on the two sides of a buried root would differ appreciably; or (3) that temperature factors could be expected to enter into the normal hydrotropic responses of roots in soils. So far as the writers have been able to determine, no experiments bearing directly on the problem of the hydrotropic responses of roots in soil have been published, and the explanations which have been assigned to hydrotropic responses in air not being applicable to soil conditions, the question arises as to whether hydrotropism is a normal response of roots growing in soil.

Methods

The findings of HENDRICKSON and VEIHMEYER (2), that a soil at the moisture equivalent, sometimes called the field coefficient, does

not lose moisture by capillary action to dry soil in intimate contact,
suggested the method used in these studies. A large sample of sandy
loam soil with an observed wilting coefficient of 7.1 per cent was air
dried to 4 per cent moisture, and one-half of the soil was then mois-
tened to 11.8 per cent. This moisture percentage appeared to be
nearly optimum for plant growth as determined by the crumbling
test; seeds germinated in samples of the soil grew readily until its
moisture content was reduced to the wilting point. When a sample
of the dry soil was packed into a cylinder, moistened from the top,
and allowed to stand for three weeks, a clear-cut and practically sta-
tionary line between moist and wet soil was observable. The soil was
removed by layers and its moisture content determined. The upper
10 cm. averaged 13.86 per cent moisture and was uniform within the
limits of error in determination. Between 10 and 16 cm. the moisture
content decreased from 13.86 to 11.9 per cent, and between 16 cm.
and the observable moist-dry line at 19 cm. the moisture content de-
creased again from 11.9 to 9.2 per cent. Below the division line the
moisture content was 5.2 per cent and dropped off in 4 cm. to the
4.0 per cent of the experimental soil. Under the conditions in the
tube, 13.86 per cent might be taken as the field coefficient, although
after three weeks there was some movement from the lower layers of
the moist soil either by greatly reduced capillary action or by vapori-
zation, until the moisture content of the soil above the dividing line
reached a minimum of 9.2 per cent. These maximum and minimum
values of 13.86 and 9.2 per cent may be compared with a calculated
moisture equivalent of 13.0 per cent, using the equation of BRIGGS
and SHANTZ (1). The moisture content of the adjoining dry soil was
also built up, obviously by vapor movement, to a value slightly
above the calculated hygroscopic coefficient of 4.83 per cent. With
the moisture percentage of 11.8 per cent which was used in these
experiments, and with most of the experiments completed in one
rather than in three weeks, clear-cut wet and dry lines such as are
shown in figures 2, 3, and 4, which had not moved appreciably from
the original point, were obtained in all experiments. We were thus
able to obtain a sharp moisture content line, with probably some
gradation over a narrow zone as the result of vapor movement from

the moist into the dry soil layer. The roots of various plants were then grown so that the effect of this steep soil moisture gradient on the direction of growth could be observed.

Experimental results

ROOTS GROWN BETWEEN WET AND DRY SOIL

In early experiments the central portion of a shallow box was filled with the moistened soil and the ends with the dry soil, except for grooves which were dug on either side, lined with 14-mesh wire cloth, and packed with the moist soil. The boxes were placed in a moist chamber and germinating seeds of peas and corn planted in the ends of the grooves. The roots of the developing seedlings were thus surrounded on three sides and the bottom by soil below the wilting coefficient, and on the fourth side by moist soil which connected to the larger area of moist soil in the center of the box. The development of plants under these conditions is illustrated in figures 1 and 2. The radicles grew straight downward through the screen wire into the dry soil and were arrested in their development. Branch roots which grew into the dry soil were checked without any evidence of bending. Branch roots which developed in the direction of the moist soil continued until by chance they grew into the dry soil. The check in the growth of the roots growing into the soil of low moisture content tended to result in profuse branching, and those branches which in their normal arrangement were pointed toward the moist soil continued the spread of the root system. Such a trial and error method resulted in some of the roots reaching the center of the flat, and with continued secondary development it might have appeared in a short time as though such roots had developed by determination rather than by accident.

ROOTS GROWN BETWEEN VERTICAL MASSES OF WET AND DRY SOIL

In a second series of experiments a shallow flat was filled with the moist soil, the seeds to be observed were placed on the surface, and the frame of a second flat was set on top of the first, filled with the dry soil, and a cover clamped in place. The soil was surrounded by heavy paraffined paper to prevent changes in moisture content and the boxes were placed in moist chambers for 6–10 days to allow the

FIG. 1

FIG 2

FIGS. 1, 2 —Fig. 1, seedling of *Pisum sativum* grown with moist soil on the left and with dry soil on the other sides and below the plant. At points indicated by the arrows the roots grew into the surrounding dry soil and were checked in their development. No hydrotropic response is shown. Fig. 2, plants of same growing from wet to dry soil without indication of hydrotropism in either primary or secondary roots. Note that while some roots bend toward the moist soil, others bend toward the dry.

roots to develop. Some of the boxes were placed vertically so that the roots would start downward between layers of moist and dry soils. Others were placed at an angle of 45° with the dry soil on the lower side of the box. Under these conditions the geotropic response of the roots would tend to cause growth into the dry soil and a hydrotropic response sufficient to overcome the effect of gravity would be shown if the roots followed the 45° angle of the moist-dry soil division line. This last experiment was intended to simulate the experiments of SACHS, but under soil conditions. Since the wilting coefficient of the soil used was 7.1 per cent and the hygroscopic coefficient approximately 5.0 per cent, the development of the roots which grew into the soil containing 4.0 per cent moisture was quickly checked. Roots which grew into the moist soil continued normally for the duration of the experiment. This situation is well illustrated in figure 3, where the secondary roots of the bean plant on the lower or dry side are short while those in the moist soil are 10 to 20 cm. long. The relative humidity of the soil air at the different moisture levels was not measured, but since the moisture percentage of the dry soil was below the hygroscopic coefficient, it may be assumed that the contained air was unsaturated with water vapor. In contrast to this situation, the air of soil at the wilting percentage would be saturated with water vapor, so that our experiments should have afforded abnormally favorable conditions for either hydrotropic or hygrotropic responses.

Some 3000 seedlings representing 26 species were planted between vertical masses of moist and dry soil, and the bending of the primary roots was observed after approximately one week of growth. Readings were made by removing very carefully the layer of dry soil and counting first the roots whose tips had bent into the dry soil; second, those which were growing vertically on the moist-dry line; and third, those roots which had turned into the moist soil. Since the roots which turned toward the soil of lower moisture content were quickly checked in their development, while those which turned into the moist soil grew several centimeters, it was difficult to obtain comparable readings for these two classes. Few, if any, of the roots which turned into the moist soil were missed in the counts, while many of those which turned in the opposite direction were so short that they

Fig 3

Fig 4

Figs. 3, 4.—Fig. 3, roots of *Phaseolus vulgaris* showing the much greater growth of secondary roots which grew by chance into the moist soil and the failure of those which turned toward the dry soil to show any hydrotropic response Fig. 4, *Cucurbita pepo*, lower one-half, and *Citrullus vulgaris* seedlings, upper one-half, showing positive and weakly positive hydrotropic responses. The strongly positive response shown by the *C. pepo* seedlings appears to be the exception rather than the normal reaction of plant roots in soil. Note that secondary roots show no hydrotropic response.

were disturbed in removing the soil and could not be accurately counted. This difficulty probably explains a noticeable tendency in all of the experiments for a slightly higher percentage of roots in the wet soil. The complete data are given in table I. Of the Gramineae used, only one species, a sample obtained as Egyptian millet and assumed to be a variety of *Setaria italica*, was definitely positive in its reaction as shown by a tendency of the roots to bend from the vertical into the moist soil. Of the legumes, two species of lima beans and one variety of *Phaseolus vulgaris* appear to have been positive, as was also *Fagopyrum esculentum* in the miscellaneous group. The data for 25 of the 30 species and varieties are considered to indicate no significant tendency for the roots to bend toward the moist soil. These 25 varieties include 11 families and 19 genera of seed plants.

A failure of the root to respond to a moisture gradient of the type used in this experiment might be considered strong evidence against the importance of hydrotropism in soils. Certainly the bending required to count as a positive response would be very little affected by the forces of gravity. On the other hand it is possible that the moisture conditions, as a result of vapor movement, were sufficiently favorable on both sides of the growing roots so that little or no hydrotropic response was evidenced. This last point was checked by noting the ability of roots to follow a wet-dry soil line at an angle of 45°.

Roots grown between wet and dry soil at an angle of 45°

The seeds for these experiments were planted in the same manner as were those for the last series, so that they were in contact on one side with dry soil and on the other with moist soil. Germination and development were not always good under these conditions and it is possible that many roots which broke through the seed coat directly into the dry soil did not make sufficient growth to give a fair test of their hydrotropic response. Nevertheless the varieties which showed some evidence of hydrotropism in the first experiments showed also some response in these tests.

The normal tendency of the roots was to grow directly downward into the dry soil. This type of response is shown in figure 2 where

TABLE I

HYDROTROPIC RESPONSE OF ROOTS GROWN BETWEEN VERTICAL
MASSES OF WET AND DRY SOIL

PLANT	TOTAL NO. PLANTS	HYDROTROPIC RESPONSE IN PERCENTAGE OF ROOTS		
		POSITIVE	NONE	NEGATIVE
Zea mays-indentata...............	177	27.0	53.2	19.8
Triticum aestivum............... .	84	19.1	60.7	20.2
Hordeum vulgare..................	93	7.5	82.8	9.7
Zea mays-rugosa..................	108	41.7	14.8	43.5
Holcus sorghum-technicus..........	165	8.5	89.1	2.4
Gramineae....................	627	20.8	60.1	19.1
Pisum sativum............... ...	182	8.5	77.5	14.0
Glycine max..................	129	19.4	64.3	16.3
Phaseolus coccineus..	84	7.1	85.8	7.1
Phaseolus vulgaris (Michigan pea)...	43	18.6	62.8	18.6
Phaseolus vulgaris (stringless green pod)........................	182	21.5	68.1	10.4
Lupinus hartwegii................	152	5.9	88.2	5.9
Vigna sinensis.................	123	13.8	73.9	12.3
Vicia sativa.....................	53	5.7	90.5	3.8
Leguminosae................	948	12.6	76.4	11.0
Beta vulgaris (mangles)...........	42	2.4	97.6	0.0
Beta vulgaris (sugar beet)..........	95	22.1	54.7	23.2
Spinacia oleracea	157	2.5	94.3	3.2
Helianthus annuus...............	142	7.0	82.4	10.6
Tragopogon porrifolius...........	61	1.6	98.4	0.0
Linum usitatissimum..............	150	6.7	79.3	14.0
Ipomoea sp.....................	128	18.0	62.5	19.5
Raphanus sativus...	249	6.3	89.5	4.2
Citrullus vulgaris................	116	20.7	58.6	20.7
Tetragonia expansa........	9	11.1	77.8	11.1
Hibiscus esculentus..............	44	20.4	61.4	18.2
Pinus sp......................	114	0.0	100.0	0.0
Miscellaneous................	1307	9.9	79.7	10.4
Fagopyrum esculentum............	295	30.8	57.7	11.5
Phaseolus lunatus...............	54	42.6	27.8	29.6
Phaseolus limensis................	59	42.4	52.5	5.1
Phaseolus vulgaris (Red kidney).....	38	47.4	42.1	10.5
Setaria italica....................	44	61.4	36.3	2.3
Miscellaneous..................	490	44.9	43.3	11.8

pea roots have grown into the dry soil, and bending toward the dry side is as common as bending toward the moist. Three other types of development are possible: (1) the roots might grow horizontally into the moist soil; (2) they might grow downward at an angle of 45°, following the moist-dry line; or (3) they might assume some intermediate position between the vertical and the 45° bending. The first classification of roots growing horizontally was not observed except in some of the grasses where horizontal growth is reasonably common. In these, as will be explained later, the direction of growth of the root depended primarily upon the orientation of the seed. The bending of the roots at the moist-dry line and their growth downward at an angle of 45°, as illustrated in the lower half of figure 4, are designated in the tables as a strong positive response. This response is comparable with that obtained in the SACHS experiment. The upper portion of figure 4 illustrates the intermediate response in which the roots of watermelon bent toward the moist soil but did not follow the moist-dry line. This response is designated in the tables as a weak positive response. Typically the roots in this group bent at an angle of 22½° from the vertical. The complete data for these experiments are given in table II.

Two species of *Phaseolus* may be considered to have shown a positive response when 16.4 and 13.2 per cent, respectively, of their roots followed the moist line. Four other species showed a weaker positive response in that from 10.5 to 32.8 per cent of their roots bent toward the moist line but did not follow it. The results with the grasses are disregarded because the normal tendency of many of the primary roots of the grass seeds, and particularly of the primary roots of maize, is to grow downward at an angle of 45°. When corn was placed with the germ toward the moist soil and the tip downward, it was found that 90 per cent or more of the roots stayed above the 45° line and grew into the moist soil. When the germ was placed toward the dry soil, the same proportion grew below the 45° line into the dry soil. When the tip of the kernel was toward the top of the box between vertical masses of wet and dry soil, 49 per cent of the radicles bent into the dry soil and 51 per cent into the wet. If we consider the seven species of legumes and the nine miscellaneous species in the experiments reported in table II, we find that: (1) there was no tend-

ency for the roots to grow into the moist soil above the 45° line; (2) with two or possibly three species there was a significant tendency to follow the 45° line; (3) not more than one-third of the species showed any evidence of a hydrotropic response under the conditions of this experiment.

TABLE II

HYDROTROPIC RESPONSE OF ROOTS GROWN BETWEEN WET AND
DRY SOIL AT AN ANGLE OF 45°

PLANT	TOTAL NO. PLANTS	RESPONSE IN PERCENTAGE ROOTS			
		NONE	WEAK POSITIVE	POSI-TIVE	INTO WET SOIL
Zea mays-indentata.....	106	63.2	....	17.5	19.3
Zea mays-rugosa...	123	69.1	....	18.7	12.2
Triticum aestivum........	65	69.2	....	27.7	3.1
Holcus sorghum-technicus..	148	100.0	0.0	0.0	0.0
Gramineae...	442	75.4	....	16.0	8.6
Pisum sativum.......	225	100.0	....	0.0	0.0
Glycine max..	188	98.9	....	1.1	0.0
Phaseolus lunatus....	167	67.2	16.4	16.4	0.0
Phaseolus limensis.......	58	58.6	32.8	8.6	0 0
Phaseolus vulgaris...	28	82.1	14.3	3.6	0.0
Phaseolus vulgaris...	144	86.8	....	13.2	0.0
Phaseolus coccineus........	76	89.5	10.5	0.0	0.0
Lupinus hartwegii..	252	100.0	0.0	0.0	0.0
Leguminosae..	1138	85.4	9.2	5.4	0.0
Helianthus annuus.....	239	100.0	0.0	0.0	0.0
Beta vulgaris.......	107	97.2	....	2.8	0.0
Spinacia oleracea	252	100.0	0.0	0 0	0.0
Linum usitatissimum..............	197	99.5	0.0	0.0	0.5
Ipomoea sp.........	61	100.0	0.0	0.0	0.0
Raphanus sativus..	239	100.0	0.0	0.0	0.0
Citrullus vulgaris...........	113	100.0	0.0	0.0	0.0
Tetragonia expansa............. .	13	100.0	0.0	0.0	0.0
Hibiscus esculentus.....•......	71	88.7	11.3	0.0	0.0
Fagopyrum esculentum............	133	89.4 ·	8.3	2.3	0.0
Miscellaneous..................	1425	97.5	1.95	0.5	0.05

ROOTS GROWING FROM MOIST INTO DRY SOIL WITH A 45°
SLOPE IN MOIST-DRY LINE

In this series of experiments the seeds were covered with 1–2 cm. of moist soil (figs. 2–4) so that normal germination and initial growth

of the radicle were obtained. After the radicles had grown vertically for 2–3 cm. they reached the line of the dry soil and their response was measured as "none" if they grew vertically into the dry soil until the growth was checked (fig. 2); as "weakly positive" if they bent perceptibly toward the moist soil but did not follow the 45° line (upper portion of fig. 4); and as "positive" if they followed the moist line (lower portion of fig. 4). Under these more favorable growing conditions, results as shown in table III were more strongly positive although no tendency was observed for the roots to grow up into the moist soil above the wet-dry line. One variety of *Phaseolus vulgaris* gave what may be considered a normal positive, hydrotropic response. Another variety of *P. vulgaris*, one of *P. limensis*, and one experiment with *Vigna sinensis* showed a positive reaction in 20 per cent or more of the roots. Among non-legumes a miscellaneous group representing six families is considered to be without measurable hydrotropic response. A group representing five families showed weak response, and a group representing three families a strong response with an average of two-thirds of the roots following the wet-dry line. In several instances, duplicate experiments have been shown separately in the data rather than averaged. It will be observed that in general the agreement is good. Two experiments with *Cucumis sativus* check closely, two with *Tragopogon porrifolius* even more closely, two with *Vigna sinensis* moderately well, but three with *Fagopyrum esculentum* showed a considerable variation so that one experiment is thrown into the group with moderate response and two into the group with positive response.

The average response in these experiments is considerably higher than in the first or second series. We consider that conditions here were more nearly normal for the development of the seedlings and more favorable for hydrotropic responses. In spite of this there appeared to be decided variations in the reaction of various species and indications of variations between varieties of the same species. Several experiments were run to check upon the possibility that, in spite of our care to maintain uniform conditions, the observed variations may have been due to chance differences in experimental technique. Three species which had shown strong positive responses in the earlier experiments and three which had shown negative responses

TABLE III

RESPONSE OF ROOTS GROWING FROM MOIST INTO DRY SOIL
WITH 45° ANGLE*

PLANT	NUMBER OF SEEDLINGS	RESPONSE IN PERCENTAGES OF PLANTS		
		No RESPONSE	WEAKLY POSITIVE	STRONGLY POSITIVE
Phaseolus lunatus.................	17	88.2	11.8	0.0
Pisum sativum....................	65	95.4	4.6	0.0
Pisum sativum....	49	96.0	2.0	2.0
Robinia pseudo-acacia......	31	87.1	9.7	3.2
Lens esculenta................ ...	57	84.2	7.0	8.8
Glycine max....	64	93.7	4.7	1.6
Leguminosae...................	283	90.8	6.6	2.6
Vigna sinensis.....	55	76.4	20.0	3.6
Vigna sinensis.	34	55.9	23.5	20.6
Phaseolus limensis................	64	42.2	32.8	25 0
Vicia sativa......................	25	68.0	16 0	16.0
Phaseolus vulgaris (Mich. pea)	46	43.5	23.9	32.6
Leguminosae...................	224	57.2	23.2	19.6
Phaseolus vulgaris (green pod).......	59	11.9	5.1	83.0
Beta vulgaris.................. ..	32	93.8	0.0	6.2
Anethum graveolens........	64	82.8	10.9	6.3
Allium cepa..........	17	88.3	11.7	0.0
Tragopogon porrifolius............	64	98.5	1.5	0.0
Tragopogon porrifolius....	56	98.2	0.0	1.8
Cucumis sativus................	44	95.5	0.0	4.5
Cucumis sativus..	19	94.7	5.3	0.0
Pinus sp.......................	31	96.8	3.2	0.0
Miscellaneous	327	93.6	4.1	2.3
Fagopyrum esculentum............	40	60.0	12.5	27.5
Raphanus sativus.................	68	64.7	26.5	8.8
Beta vulgaris.................. ..	61	39.3	32.8	27.9
Hibiscus esculentus...............	32	62.5	21.9	15.6
Citrullus vulgaris....., ...	59	54.3	22.0	23.7
Miscellaneous.	260	56.2	23.1	20.7
Setaria italica....................	54	22.2	24.1	53.7
Fagopyrum esculentum........ ..	62	30.7	14.5	54.8
Citrullus vulgaris	37	8.1	16.2	75.7
Fagopyrum esculentum............	55	10.9	5.5	83.6
Cucurbita pepo................. ..	25	8.0	16.0	76.0
Miscellaneous...................	233	16.0	15.3	68.7

* Seedlings grew through 1.5 cm. of moist soil into dry soil lying with 45° slope. "No response" grew straight into dry soil; "weak positive" response turned toward moist soil at angle of about 22° but grew into dry; "positive" response followed line of moist soil at 45° angle.

were planted in pairs in three flats. Each flat was planted in alternating rows, one-half to a positively and one-half to a negatively responding species. The data in table IV show that there was unquestionably a pronounced variability in the reaction of these species. The differences when the two plants were grown in the same flat were comparable with the differences obtained in the other experiments where ordinarily two varieties were planted in each flat, but they were chosen on the basis of convenience in seeding and reading the results rather than on the basis of the reaction of the plants.

TABLE IV

VARIATIONS IN HYDROTROPIC RESPONSE
FLATS PLANTED WITH ALTERNATING ROWS OF TWO SPECIES AND HELD
AT ANGLE OF 45°

PLANT	FLAT NO.	PERCENTAGE OF TOTAL SHOWING		
		No RESPONSE	WEAKLY POSITIVE	STRONGLY POSITIVE
Tragopogon porrifolius............	1	98.5	1.5	0.0
Citrullus vulgaris.................	1	8.1	16.2	75.7
Cucumis sativus........	2	95.5	0.0	4.5
Fagopyrum esculentum., ...	2	10.9	5.5	83.6
Cucumis sativus..................	3	94 7	5.3	0.0
Cucurbita pepo...................	3	8.0	16.0	76.0

Summary

1. Seven thousand seven hundred sixty-three seedlings representing 29 genera and 14 families have been grown in a great number of experiments to determine the hydrotropic response of primary roots of seedlings growing on a steep soil moisture gradient. The steep gradient has been obtained by placing in contact a layer of soil with a moisture percentage below the hygroscopic coefficient and a layer with a moisture percentage slightly below the moisture equivalent or field coefficient. This gradient was intended to provide optimum conditions for a hydrotropic response, and is steeper than will ordinarily be encountered in the field where the wilting percentage normally represents the low moisture content of the areas into which roots might grow. Under these conditions the growth of seedlings in

the moist soil has been normal and a sharp moist-dry line between
the two soil layers has been maintained over a period of one to two
weeks. In contrast to the normal growth of roots in the moist layer,
growth has been completely checked in roots which have penetrated
more than approximately 1 cm. into the dry soil.

2. The primary roots of many plant species have shown no tend-
ency to bend when growing from the moist soil layer with available
moisture into the dry soil with insufficient moisture to maintain
growth. The typical response in this group of plants has been a
growth along the lines determined by the position of the seed and the
normal response to gravity, until the root has grown into the dry
soil. Under these conditions branching was stimulated in the por-
tions of the root remaining in the moist soil. These branches grew
normally outward and down without showing hydrotropic response,
and continued in the moist soil or passed from it into the dry soil, de-
pending upon their initial direction of growth. Those which grew
into the dry soil, with favorable food conditions, again formed
branches which repeated the response of the earlier roots.

3. A second group of plants showed weak hydrotropic responses.
A small percentage of the primary roots bent at an angle of 45° from
the vertical and followed a moist-dry soil line. Normally a some-
what larger percentage showed some bending toward the more moist
soil but did not follow the line at an angle of 45°.

4. A third and smaller group of plants showed responses in soil
comparable with those obtained in air with the SACHS experiment,
with from one-half to three-fourths of the roots following the moist
soil at an angle of 45° and another fraction bending toward the mois-
ture but not following the 45° line. Secondary roots as a group
showed no clear-cut hydrotropic response in any of these experi-
ments.

5. The data indicate that hydrotropism in roots is by no means
comparable in distribution and intensity of reaction with geotropism
in roots and stems or with phototropism in stems. The results with
several species of the Cucurbitaceae and Leguminosae suggest that
hydrotropic responses may depend upon a genetic factor present in
some plants and absent from closely related species or varieties.

6. Apparently these results demonstrate the possibility of a re-

sponse in soil similar to that which has been obtained in the air. On the other hand, they seem to show equally clearly that hydrotropism is not a universal and probably under field conditions not a common plant response.

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LITERATURE CITED

1. Briggs, L. J., and Shantz, H. L., The wilting coefficient for different plants and its indirect determination. Bur. Plant Ind. Bull. 230. 1–77. 1912.
2. Hendrickson, A. H., and Veihmeyer, F. J., Soil moisture conditions in relation to plant growth. Plant Physiol. 2:71–81. 1927.
3. Hooker, H. D. Jr., Hydrotropism in roots of *Lupinus albus*. Ann. Bot. 24: 265–283. 1915.
4. Molisch, H., Untersuchungen über den Hydrotropismus. Sitzungsber. K. K. Akad. Wien 88:897–943. 1883.
5. Sachs, Julius F., Ablenkung der Wurzel von ihrer normalen Wachsthumsrichtung durch feuchte Körper. Arbeit. Botan. Inst. Würzburg. 1:209–222 (1874). 1872.

EFFECTS OF NITROGEN SUPPLY ON RATES OF PHOTOSYNTHESIS AND RESPIRATION IN PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 471

KARL C. HAMNER

Introduction

There is a conspicuous lack of recorded results of experiments to determine the effect of variations in the nutrient supply of plants on their photosynthetic and respiratory rates. SPOEHR and McGEE (7), working with excised leaves of *Helianthus*, found that if the petioles were immersed in nutrient solutions containing certain amino acids the respiratory rate was increased. They also studied the photosynthetic rates of excised leaves after varying periods of exposure to darkness, and found some correlation between the respiratory rates and the photosynthetic rate. GREGORY and RICHARDS (3) grew barley plants with various deficiencies of nutrient supply along with others with complete nutrient solution. They measured the respiratory rates of single leaves removed from the plants at various stages in growth and found that nitrogen deficiency had little or no effect on the CO_2 assimilation rate and a slightly depressing effect on the respiratory rate. BRIGGS (1), using excised leaves, also studied the effect of mineral deficiencies on the assimilation rate.

The purpose of the work here described was to determine the rates of photosynthesis and respiration of leaves attached to the plant, and of whole plants grown under different conditions of nitrogen nutrition, when such plants were subjected to various changes in the relative planes of nutrient supply. For example, if plants had been grown for a period with very low nitrate supply, so that they became relatively high in reserve carbohydrates, their photosynthetic and respiratory rates were studied first under the low plane of nitrate supply, then they were shifted to a high plane of supply and these same rates continuously determined. Similarly, tests were

made of plants on a high plane of nitrate supply and then shifted to a low one, with various intermediate conditions, as seemed desirable at any given time.

I. Experimental work on tomato

METHODS AND APPARATUS, ETC.

Tomato plants were grown in the greenhouse in rich soil until they were about 6 inches high, at which time they were removed from the pots, the roots washed clean, and most of them clipped off close to the stem. The bases of the plants were then stuck 1–3 inches deep in clean moist quartz sand. New roots were soon developed. The nutrient solutions used throughout these experiments were the same as those described by NIGHTINGALE (6). The one containing nitrate was generally supplied for several days or longer following transplanting; but later all nitrates were omitted, and as a result the plants usually accumulated large reserves of storage carbohydrates as they continued to grow in the greenhouse.

At the beginning of each experiment six pots of plants were selected for uniformity and the relative vegetative or non-vegetative condition of the plants carefully noted. A general estimate of the reserves of nitrates and carbohydrates was determined by microchemical examination of the fresh tissue. The plants were then placed in a room which was held at constant humidity and temperature. Four 1000 watt tungsten bulbs placed at a distance of about 30 inches from the plants were used as the light source. An alternation of ten hours of exposure to light and fourteen hours in darkness was maintained throughout any given experiment.

For the detailed studies of carbon dioxide uptake and output, two leaves, one each from plants in separate pots, were selected. These will be referred to as the experimental leaves. They were green, approximately alike in appearance and area, and located as near the top of the plant as was possible, consistent with the ability to secure those which were fairly well matured and had attained practically full size. The leaf area was determined by printing an outline of the leaf on blueprint paper and later measuring the area by means of a planimeter. The light intensity was measured with a McBeth il-

luminometer. Adjustments were made so that the light intensity at the surface of each experimental leaf was the same at the beginning of each experiment.

The work with tomatoes was carried out in 1933. Since that time several changes have been made in the detail of the apparatus used. In its modified form it has been described by MITCHELL (4). The type of leaf chamber used, the method of sealing the plants into the chambers, the movement of gases through the chambers, and the methods of analysis of CO_2 are essentially the same as those described. In this early work, however, tungsten lamps were used as the light source. The concentration of CO_2 in the gas stream entering the leaf chamber was the same as in outside air. Under the light from tungsten bulbs there was a tendency for the leaf chambers to become heated, and in order to maintain the temperature inside the chamber equivalent to that outside it, the air in the chamber was cooled by passing it from the chamber by means of pumps (4) to coils in a cold water bath and then back to the chambers. There was no evidence in this work that the addition of nitrates to the plants used increased the rate of photosynthesis. This might be accounted for on the basis of some of the methods employed. The leaves inclosed in the gas chamber removed from 30 to 60 per cent of the CO_2 from the air stream passing over them and it may be that CO_2 was not present in sufficient quantities to permit an increase in the photosynthetic rate. It was also evident that the light source was not sufficiently intense nor of proper quality for growth and development of the plants, as shown by the fact that all the plants etiolated and lost some of their lower leaves. It was impossible to continue any given experiment for more than four or five days because the plants became so etiolated that they were no longer fit for use, or the leaves in the experimental chambers yellowed and died. It may be that if the proper light source were used and the CO_2 of the air stream in the chamber maintained at the same concentration as in atmospheric air, there would be an increase in the photosynthetic rate. Such a result was obtained, as is detailed later in the paper in the experiments on wheat.

EXPERIMENT I.—Six pots of plants were placed in the constant temperature room at 8 A.M. on July 28, 1933; two leaves were

selected on plants in different pots and sealed in the chambers. The circulation of gas through the chambers was started and the plants were illuminated until 4 P.M., which marked the close of the first light period. The plants were then in darkness for fourteen hours and this alternation of light and darkness continued throughout the experiment. Measurements of the CO_2 output of the experimental leaves were made throughout each period of darkness, records being taken at the end of every two hours. Nitrates were applied to three pots, including one of the pots with a plant bearing an experimental leaf, at the end of the second light period (in this case at 3 P.M., July 29).

The light intensity at the surface of each experimental leaf was 850 foot candles at the beginning of the experiment. At the end of the experiment it was 820 foot candles at the surface of the leaf on the plant which received no nitrate in the nutrient solution, and 850 foot candles at the surface of the other experimental leaf. The fall in light intensity in the former case was doubtless due to deterioration of the lamps used. The temperature of the room was 22° C. and the relative humidity was maintained at 75 per cent.

The plants when taken from the greenhouse were extremely high in reserve carbohydrates. All the cells of the mesophyll and palisade were filled with starch, which was abundant also throughout the parenchymatous tissues of the stem except at the very tip. The plants were hard and woody and showed an almost complete lack of nitrates. The changing composition of the plants was followed as closely as possible microchemically during the entire duration of the experiment. Those plants which were not inclosed in the small chamber and to which the nitrates were added remained high in carbohydrates, although they became slightly etiolated. The upper internodes lengthened, and growth at the tip took place rapidly. Yellowing and death of the lower leaves also occurred. The leaf inclosed in the chamber showed progressive yellowing from the margin of the leaflets inward during the course of the experiment and at the close was about to fall from the plant.

Those plants to which nitrates were added, when compared with those which received no nitrogen, appeared slightly greener, and both stem and leaves enlarged more than in the case of the minus

nitrogen plants. These also etiolated somewhat and lost their lower leaves. In four to six hours after nitrates had been added tests for nitrates were positive well up in the plants, and in twenty-four hours nitrates were in abundance throughout. Nitrites became apparent about two hours after the nitrates were detectable. After fourteen hours the plus nitrate leaf was noticeably greener than the minus nitrogen one, and had enlarged appreciably.

TABLE I

EXPERIMENT I: RESPIRATION IN TOMATO PLANT

INTERVAL OF READING (EACH OF 2 HOURS' DURATION)	CO_2 RESPIRED PER SQ. METER OF LEAF AREA PER HOUR (MG.)			
	1ST DARK PERIOD	2ND DARK PERIOD	3RD DARK PERIOD	4TH DARK PERIOD
	PLUS NITRATE			
1............	167	84	171	....
2........	119	84	163	143
3..........	116	92	163	86
4......	....	95	....	86
5...............	99	....	155	86
6...............	73	109	131	73
	MINUS NITRATE			
1............... ..	203	120	121	....
2......	155	104	112	73
3...............	131	112	118	86
4...... ..,.,....	129	99	111	86
5...............	114	94	104	86
6............. ..	114	87	104	87

The results of the respiratory measurements are given in table I. It is obvious that following the addition of nitrates, the respiration rate is appreciably increased.

EXPERIMENT II.—This experiment was begun in the constant temperature room on August 2, 1933. The same details of procedure were followed as before. When placed in the room the plants were nitrate-free but were slightly lower in reserve carbohydrates than those of the previous lot. They appeared slightly greener and the

stem tips were softer. They were high in reserve carbohydrate, however, and behaved very similarly to those of experiment I. During the course of the experiment they etiolated somewhat and lost their lower leaves. The experimental leaf of the plant to which no nitrates had been added became yellow as before and was ready to fall from the plant at the close of the experiment. Those plants to which

TABLE II

EXPERIMENT II: RESPIRATION IN TOMATO PLANT

INTERVAL OF READING (EACH OF 2 HOURS' DURATION)	CO_2 RESPIRED PER SQ. METER OF LEAF AREA PER HOUR (MG.)		
	1ST DARK PERIOD	2ND DARK PERIOD	3RD DARK PERIOD
	PLUS NITRATE		
1........	202	144	199
2	148	125	165
3...............	148	114	165
4	119	119	153
5..	125	94	165
6......	114	95	153
	MINUS NITRATE		
1..........	254	188	126
2..	151	126	108
3...	126	126	100
4................	126	107	108
5...............	125	115	100
6...	123	126	111

nitrates were added became noticeably greener and enlarged considerably.

Nitrates in slight amounts made their appearance in the upper parts of the plants in twelve hours after their application. Nitrites were evident several hours after the nitrates appeared in the tops, but were present only in very small amounts.

The results of the respiratory measurements are given in table II. In general the plants responded more slowly to nitrate application

than did the plants of the previous experiment. As compared with the results of experiment I, there was less increase in the rate of respiration after the application of nitrates and the response was delayed for a longer period.

EXPERIMENT III.—The plants were removed to the constant temperature room on August 8, 1933 at 8 A.M. and the measurements

TABLE III

EXPERIMENT III: RESPIRATION IN TOMATO PLANT

INTERVAL OF READING (EACH OF 2 HOURS' DURATION)	CO_2 RESPIRED PER SQ. METER OF LEAF AREA PER HOUR (MG.)		
	1ST DARK PERIOD	2ND DARK PERIOD	3RD DARK PERIOD
	PLUS NITRATE		
1................	182	....	200
2............ ..	133	137	168
3.	139	128	159
4...............	139	111	168
5...	135	106	166
6................	116	90	164
	MINUS NITRATE		
1................	190	143	....
2................	151	136	78
3................	147	133	102
4.	149	88	89
5.....	147	....	71
6................	125	100	....

started at 4 P.M. as before. The procedure followed was the same as that used in the previous two experiments. The plants used might be described as being intermediate in condition and response between the plants of the first and the second experiments. They were slightly taller than those of the previous two lots, but the number and size of the leaves were about the same. They were not so high in reserve carbohydrates as the first lot of plants, but higher than the second. Upon the application of nitrates the plants turned

greener and enlarged as before. Nitrates were present in small amounts after six to eight hours, and nitrites appeared a few hours later.

The results of the respiratory measurements are given in table III. In general the results occupy an intermediate position between those obtained in the first and second experiments.

TABLE IV .

EXPERIMENT IV: RESPIRATION IN TOMATO PLANT

INTERVAL OF READING (EACH OF 2 HOURS' DURATION)	CO_2 RESPIRED PER SQ. METER OF LEAF AREA PER HOUR (MG.)		
	1ST DARK PERIOD	2ND DARK PERIOD	3RD DARK PERIOD
	PLUS NITRATE		
1................	63	....	162
2................	60	64	173
3................	55	68	184
4............. ...	48	64	....
5................	52	75	160
6..............	32	72	139
	MINUS NITRATE		
1...........	81	....	18
2.	81	37	18
3.	67	24	10
4................	67	29	....
5...	70	32	10
6..	56	36	0

EXPERIMENT IV.—Except for the fact that they were slightly larger, contained more lignified tissue, and had a slightly hollow stem, the plants used in this experiment were similar to those used in the first experiment. They were extremely high in reserve carbohydrates; and as in the first experiment, all of the mesophyll and palisade cells of the leaves were filled with starch which was abundant also throughout the parenchymatous tissues of the stem except at the very tip. No reserves of nitrates were apparent.

The plants were placed in the constant temperature room on August 15, 1933 and treated as usual. Nitrates were present throughout the plants within three hours after their application in the nutrient solution, and nitrites appeared two hours later. The leaves of the plants to which nitrates were added became greener than the others within eight to ten hours after the application of nitrates.

Results of the gas analyses are given in table IV. The results are similar to those in experiment I except that the increase in rate of respiration is greater.

SUMMARY

On the basis of the foregoing data it is evident that the composition of any given plant is in a marked degree correlated with its respiratory activity. Thus tomato plants very high in carbohydrates but low in their nitrate or soluble nitrogen content may have a relatively low respiratory rate, whereas one which is higher in its nitrate content will have an appreciably higher one. Or any given plant high in its carbohydrate reserves will have its respiratory rate increased if nitrates are supplied to it in abundance, other conditions remaining the same. Although all the plants used in the various experiments were very high in reserve carbohydrates, a relatively slight change in the amount of this reserve had a great effect upon the time required for nitrates to make their appearance in the tops of the plants after they had been applied in the nutrient solution. In every case the addition of nitrates to plants high in reserve carbohydrates caused an increase in respiration, the greater such reserve the more nearly immediate the response and the greater in degree. In the case of plants extremely high in reserve carbohydrates the response was almost immediate and very marked. In the first and last experiments respiration of the plants to which nitrates had been added increased to three times that of the plant which received no nitrate. In the case of plants slightly lower in reserve carbohydrates the response was not so great, nor did it occur at once. In the second experiment a response was noticeable only after twenty-four hours, and the increase in respiration was about 100 per cent. In the third experiment the response became evident in twenty-four hours, and the total increase was 120 per cent. In each case the increase in rate

of respiration seemed to take place subsequent to the appearance of nitrates in the top of the plant; in no instance did it occur prior to such appearance, whatever the length of time elapsing after their application in the nutrient solution.

II. Experimental work on wheat

It was desired to supplement the work on tomatoes, since the measurements were made on single leaves instead of on entire plants. Also it was apparent that the plants would not accumulate reserves of carbohydrates nor maintain those reserves already present when they were placed under Mazda lights. In the work on wheat it was found possible under artificial illumination to grow plants which would continue to increase in size and in dry weight. By varying the nitrogen supply in the nutrient solution, plants either very high or very low in reserve carbohydrates, as was desired at any particular time, were obtainable. It was possible also to obtain separate quantitative measurements on the respiration of roots and of tops and also measurements of the CO_2 assimilation of the entire tops.

The apparatus finally devised and used in this work is that described in detail by MITCHELL (4). That part of the apparatus described for experimentation with entire plants was designed for these experiments.

Wheat grains of the Marquis variety, secured from Corvallis, Oregon, were sterilized in 0.25 per cent uspulun for five minutes and planted in sterilized quartz sand. In each experiment 50 grains were planted per pot. Six-inch clay pots and pyrex beakers of 1500 cc. capacity and with necessary outlets were used as containers. These were placed in the constant temperature room, watered with distilled water for the first week, and exposed to a light intensity of about 300 foot candles. At the end of the first week the pots containing the plants were placed in a pan of distilled water so that the water level reached the upper surface of the sand in the pot, and a little of the sand around the base of the seedlings was washed away. The ungerminated grains were picked out. All seedlings in excess of fifteen per pot were removed in entirety, care being taken that those remaining be as uniform in size as possible.

The pots were then placed about 30 inches from the light in posi-

tions such that the plants in each pot were exposed to an intensity
of about 800 foot candles for a period of twelve hours. The light was
then turned off and the plants were in darkness for the remaining
twelve hours of each day.

The nutrient solutions employed throughout these experiments
were the same as those used by NIGHTINGALE (6). One of these con-
tained no nitrate, the other contained nitrate in abundance. For
four days following the time when the cultures were placed in full
light they were supplied with the nutrient solution containing no
nitrate, diluted one-half with distilled water. For at least two days
more all the cultures received the same solution, undiluted. Subse-
quent treatment of the plants varied in the several experiments con-
ducted. In all of them, however, the plants were sealed in the cham-
bers at twelve to fourteen days after the sowing of the grains. After
sealing in the chambers all the cultures were supplied with minus
nitrate solution for two days more. Then, commencing at the end of
the light period of the third day, half of the plants received the same
minus nitrate solution and half the plus nitrate solution. This treat-
ment was continued throughout any given experiment.

During the first two days in the chambers, readings were made of
the CO_2 uptake during the last four hours of the light period, and
CO_2 output during the first four hours of the dark period. At the end
of the light period of the third day the plus nitrate nutrient solution
was applied to one-half of the cultures, and readings on CO_2 output
of both the plus and minus nitrate cultures made for the following
four-hour dark period. Subsequent readings of uptake and output
were made for such periods and at such times as already stated for
the first two days the plants were in the chambers.

It was found that essentially the same values were obtained for
the uptake and output over a given time interval, whether the read-
ings were made at the beginning or the end of a given light or dark
period. For convenience in making measurements and in recording
results, only readings for the last four hours of the light period and
the first four hours of the dark period of each day are given.

In each experiment thirty groups of plants were used. Six of these
were the pyrex beakers mentioned, and twenty-four were the 6-inch
clay pots. The plants in the six beakers were used for the measure-

ments of CO_2 uptake and output. At the time when nitrates were added to some of the plants, those in eight of the clay pots were harvested and constituted the initial sample. Such plants, of course, had received no nitrate. Eight of the remaining clay pots and three

TABLE V

EXPERIMENT V: QUANTITATIVE DETERMINATIONS OF CO_2 UPTAKE AND OUTPUT BY YOUNG WHEAT PLANTS

AGE OF CULTURE IN DAYS	CO₂ ASSIMILATED PER 100 PLANTS DURING 4-HOUR INTERVAL OF PERIOD OF EXPOSURE TO LIGHT (MG.)	CO₂ LIBERATED PER 100 PLANTS DURING 4-HOUR INTERVAL OF PERIOD IN DARKNESS (MG.)		CO₂ ASSIMILATED PER 100 PLANTS DURING 4-HOUR INTERVAL OF PERIOD OF EXPOSURE TO LIGHT (MG.)	CO₂ LIBERATED PER 100 PLANTS DURING 4-HOUR INTERVAL OF PERIOD IN DARKNESS (MG)	
		ROOTS	TOPS		ROOTS	TOPS
	RECEIVING NO NITROGEN IN NUTRIENT SOLUTION					
14..............	186	32 4	15 2	164	33 2	11 9
15..............	186	29 8	15 5	152	30 6	14.9
16..............	186	....	....	154	....	....
	AFTER ADDITION OF NITRATES TO NUTRIENT SOLUTION SUPPLIED TO SOME OF THEM					
	PLUS NITRATE			MINUS NITRATE		
16..	....	41 7	15 5	.. ..	?9 8	13.6
17.....	221	42.1	24.2	133	29 6	13.7
18..............	290	35 6	18 0	140	30.0	14.0
19..............	... .	21.0	15 2	....	29 8	14 1
20............	310	31.9	21 3	154	....	....
21............	290	....	....	140	30.8	11.1
22............	279	35 1	21.5	133	....	....
23............	279	....	....	128	31.9	10 4
24............	....	39.0	22.3	....	....	....
25............	234	....	....	138	30.1	8.9
26............	234	41.0	23.8	140	....	..... .
27............	....	....	....	....	30.4	9.1
28............	226	42.8	29.0	168	29.3	8.7

of the beakers were then supplied with nitrates in the nutrient solution and the others were continued with the minus nitrogen nutrient solution. At the end of the experiment all the plants in the clay pots were harvested and constituted the final sample.

The results of three separate experiments are given here, since they are representative of several which show the same results. The first experiment extended from the time the plants were fourteen days old until they were twenty-eight days old. At the age of fourteen days none of the plants showed definite visible signs of nitrogen deficiency except that they were a light green. At the end of the experiment, however, those plants which had been supplied with no nitrogen in the nutrient solution showed definite injury. The lower leaf was usually yellow throughout its length and nearly dead; often the second leaf was dead at the tip and yellow nearly half the dis-

TABLE VI

EXPERIMENT V

	DRY WEIGHT PER 100 PLANTS (GM.)			FRESH WEIGHT PER 100 PLANTS (GM.)		LEAF AREA PER 100 PLANTS (SQ. DM.)
	ROOT	TOP	TOTAL	ROOTS	TOPS	
Initial sample......	1.400	1 627	3.027	20.90	12.1	5.00
Plus nitrate plants 14 days later........	1 803	4.352	6.155	37.1	34 9	13.06
Minus nitrate plants 14 days later.....	2.430	2.168	4.598	47.5	16 4	6.04

tance toward the base of the leaf. The rest of this leaf and the rest of the leaves were a yellowish green in color. Those plants to which nitrates had been added were a deep green except for the lowest leaf which often was partially dead. The other leaves were a fresh green color and continued to increase rapidly in size, more than doubling in area by the end of the experiment. The significant data are given in tables V–VI.

A second experiment of the same general nature as the first was also performed. All conditions were kept as nearly the same as possible, except that readings on uptake and output were begun when the plants were twelve days old instead of fourteen. This was in order that the plants might not have undergone as much injury from nitrogen deficiency as under the first experiment. The data are given in tables VII–VIII.

A third experiment was carried out to eliminate some of the variations apparent in the preceding two experiments and to obtain data concerning the changes in root volume and root area. The root volume was obtained by placing the clean fresh roots in water and

TABLE VII

EXPERIMENT VI: QUANTITATIVE DETERMINATIONS OF CO_2
UPTAKE AND OUTPUT BY YOUNG WHEAT PLANTS

AGE OF CULTURE IN DAYS	CO_2 ASSIMILATED PER 100 PLANTS DURING 4-HOUR INTERVAL OF PERIOD OF EXPOSURE TO LIGHT (MG.)	CO_2 LIBERATED PER 100 PLANTS DURING 4-HOUR INTERVAL OF PERIOD IN DARKNESS (MG.)		CO_2 ASSIMILATED PER 100 PLANTS DURING 4-HOUR INTERVAL OF PERIOD OF EXPOSURE TO LIGHT (MG.)	CO_2 LIBERATED PER 100 PLANTS DURING 4-HOUR INTERVAL OF PERIOD IN DARKNESS (MG.)	
		ROOTS	TOPS		ROOTS	TOPS
RECEIVING NO NITROGEN IN NUTRIENT SOLUTION						
12............	178	32.5	20.5	178	31.9	14.6
13............	189	....	....	178	....	....
AFTER ADDITION OF NITRATES TO NUTRIENT SOLUTION SUPPLIED TO SOME OF THEM						
	PLUS NITRATE			MINUS NITRATE		
13............	....	51.9	17 5	....	31.9	14.9
14............	201	40.4	17.3	165	33.5	14.3
15....	247	32.4	19.1	161	34.6	13.3
16.........	258	34.6	20.5	149	....	....
17........	266	38.6	24.5	160	31.4	14.1
18............	....	....	....	....	32.4	12.8
19............	279	38.6	25.2	146	....	....
20............	....	41.2	26.8	....	31.9	12.2
21............	247	....	....	160	....	....
22............	237	45.2	31.1	145	31.9	12.8
23............	....	....	....	....	....	....
24............	235	47.9	31.9	142	30.6	8.5

measuring the volume of the displaced liquid. The root area was an approximation calculated from the area covered by the projection of the root system on to a plane surface. The latter area was obtained with an apparatus described by MITCHELL (5). The clean fresh roots were spread out on a plate glass and the area covered

by their shadows measured as described. This area was multiplied by 3.14 to give root area. All conditions were kept as nearly the same as those of the first experiment as possible, except that the plants were watered with minus nitrogen nutrient solution diluted one to ten with distilled water during the first week. Screens were placed behind the chambers in such a manner that some of the light passing through or around them might be reflected back, thus giving a more

TABLE VIII

EXPERIMENT VI

	DRY WEIGHT PER 100 PLANTS (GM)			FRESH WEIGHT PER 100 PLANTS (GM)		LEAF AREA PER 100 PLANTS (SQ DM)
	ROOT	TOP	TOTAL	ROOTS	TOPS	
Initial sample	1 160	1 488	2 648	20 8	11 66	4 47
Plus nitrate plants 12 days later	1 380	3 540	4 920	35 0	31 8	10 82
Minus nitrate plants 12 days later	2 00	2 04	4 04	45 0	15 8	4 86

nearly uniform lighting of the entire chamber and of the plants inside it. The significant data are given in tables IX and X.

It will be obvious at once that there was some fluctuation in the readings, each of which is the average of three separate pots of plants (45 plants in all), but there are definite and marked trends, which are evident in all the experiments. It is not yet possible to account precisely for some of the variations in CO_2 uptake and output. They may have been due to minor fluctuations in the relative humidity or to other seemingly minor variations in environmental conditions. Great care was taken to keep these factors as nearly uniform as possible, but at times they were not absolutely constant. As already stated, screens were placed behind the plants in the last experiment in order to obtain a more nearly uniform lighting of the entire chamber and of the plants. This was done because in the previous experiments a slight fog had accumulated on one side of the chambers and it was thought that this might reflect varying amounts of light according to its density. The readings in the last experiment are slightly more uniform than those of the previous

experiments. All the experiments agree in their general trends and the trends shown are definite and of sufficient magnitude to remain unobscured and valid in spite of the variations shown.

TABLE IX

EXPERIMENT VII: QUANTITATIVE DETERMINATIONS OF CO_2 UPTAKE AND OUTPUT BY YOUNG WHEAT PLANTS

AGE OF CULTURE IN DAYS	CO_2 ASSIMILATED PER 100 PLANTS DURING 4-HOUR INTERVAL OF PERIOD OF EXPOSURE TO LIGHT (MG.)	CO_2 LIBERATED PER 100 PLANTS DURING 4-HOUR INTERVAL OF PERIOD IN DARKNESS (MG.)		CO_2 ASSIMILATED PER 100 PLANTS DURING 4-HOUR INTERVAL OF PERIOD OF EXPOSURE TO LIGHT (MG.)	CO_2 LIBERATED PER 100 PLANTS DURING 4-HOUR INTERVAL OF PERIOD IN DARKNESS (MG.)	
		ROOTS	TOPS		ROOTS	TOPS
RECEIVING NO NITROGEN IN NUTRIENT SOLUTION						
14..........	167	29.6	15.1	173	30.9	14.0
15..........	175	29.0	14.7	172	29.8	13.8
16..........	178	....	....	168	....	....
AFTER ADDITION OF NITRATES TO NUTRIENT SOLUTION SUPPLIED TO SOME OF THEM						
	PLUS NITRATE			MINUS NITRATE		
16..........	....	40.0	16.1	....	29.6	13.5
17..........	219	45.0	16.6	170	30.1	14.1
18..........	220	30.9	15.8	166	30.0	13.7
19..........	260	32.1	17.1	166	31.2	13 3
20..........	280	34.2	17.0	176	30.8	13.5
21..........	250	34.0	18.6	174	29.9	12.8
22..........	245	36.0	20.5	168	30.4	13.1
23..........	260	36.1	23.9	176	29.8	12.5
24..........	260	37.8	25.0	168	29.8	11.8
25..........	262	39.1	25.0	176	30.7	10 8
26..........	....	41.6	26.2	....	30.6	10 6
27..........	256	42.7	26.9	167	31.2	10.1
28..........	250	45 3	28.7	170	30.5	9.2
29..........	245	50.8	30.9	172	30.2	8.7
30..........	238	....	....	174	....	....
31..........	240	52.9	32.4	170	31.8	8.8

CULTURES TO WHICH NO NITRATE WAS SUPPLIED.—Perhaps the most striking result is the fact that the minus nitrate plants, although part of the leaves died and those that remained alive became

yellow to yellowish green and increased but little in size, yet were
able to assimilate relatively large quantities of CO_2. That there is
not an error of measurement involved is confirmed by the figures on
the dry weight of these plants. These showed an increase comparable
with the amount of CO_2 absorbed over the amount lost in respiration.
This must mean, therefore, that on the basis of unit area of the leaf
the rate of carbon fixation has undergone practically no decrease
during the experiment. It is not possible to say with precision just
how many living active cells capable of photosynthesis there may

TABLE X

EXPERIMENT VII

	DRY WEIGHT PER 100 PLANTS (GM)			FRESH WEIGHT PER 100 PLANTS (GM)		LEAF AREA PER 100 PLANTS (SQ DM)	ROOT AREA PER 100 PLANTS (SQ DM)	ROOT VOL- UME PER 100 PLANTS (CC)
	ROOT	TOP	TOTAL	ROOTS	TOPS			
Initial sample	1 20	1 60	2 80	20 5	12 05	4 17	12 62	27 7
Plus NO₃ plants 16 days later	1 87	5 28	7 15	36 3	45 32	14 77	19 77	40 1
Minus nitrate plants 16 days later	2 40	2 48	4 88	46 04	15 5	4 51	21 54	34 5

have been nor what their exact volume was, but that those which
were capable of functioning continued to do so at a fairly rapid rate
there is no doubt. The relatively large capacity of these cells to fix
the quantity which they did may well furnish a definite basis for
understanding why plants which are small because of nitrogen star-
vation are still able to accumulate large amounts of carbohydrate or
carbohydrate derivatives, either as reserve foods or structural ma-
terials. It may also aid in an interpretation of the behavior of some
species when subjected to short photoperiods.

The total amount of CO_2 output of the tops during the periods of
the experiment showed a quantitative decrease. This is in accord
with the results of GREGORY and RICHARDS (3). Thus the rate of
respiration per unit of area would at first seem to be markedly less,
but actually may not have been so, since some of the apparently liv-

ing tissues measured may actually have been dead. Again the results are of such nature that valid deductions are possible.

In contrast to the relatively small increase in the tops of these minus nitrate plants, the roots gained appreciably in volume, area, and more than 100 per cent in dry weight. Since the total amount of CO_2 given off remained about the same despite the increase in volume of the root and since there was virtually no dead tissue present, the respiratory rate showed an actual decrease. It is apparent, therefore, that the elaborated materials from the tops were in this case used in extension of the root system and the building up of a dry weight reserve. This is in marked contrast to the roots from the plus nitrate cultures, as will be discussed later.

CULTURES TO WHICH NITRATE WAS SUPPLIED.—The tops of these plants began to increase markedly in size after the application of nitrate. They were a fresh bright green and by the end of the experiment had more than doubled in dry weight. The volume of CO_2 absorbed increased markedly during the first five days, then declined somewhat despite the continued increase in leaf area, and finally leveled off to an amount slightly in excess of that before the nitrate was applied. Thus it is obvious that the rate of photosynthesis per unit of leaf area at first markedly increased and then later actually decreased. It was thought that most of the leaves which expanded during the experiment might already have been differentiated when nitrate was added so that there was little or no increase in the number of stomata operative. That this was not true was shown by stomatal counts. It may be that this decrease in the rate of CO_2 uptake is associated with the increasing age of the plants. GREGORY and RICHARDS (3) claim that in barley there is a decrease in the photosynthetic rate of all of the leaves as the plant grows older. There was no such decrease in the minus nitrate plants, however. It is possible that at the beginning of the period there was a metabolizable carbohydrate reserve in the leaves which soon became used up in expansion of the volume of leaf and that the newer chemical relations set up within the leaf were not favorable to increased photosynthesis. There was at all times an abundance of CO_2 in the surrounding atmosphere so that it did not become a limiting factor. The results, which were obtained repeatedly in these experi-

ments, were not wholly in accord with what might have been expected on the basis of many other results already recorded in the literature. This difference, however, makes the results obtained all the more interesting, for they certainly call for a further chemical investigation of leaves in relation to their capacity for CO_2 uptake and fixation.

In general the tops of the plants showed a gradual rise in CO_2 output as the leaves expanded. Except in one instance, there was no abrupt rise and fall, and in that instance there was a high initial carbohydrate content of the leaves. On the basis of unit of leaf area, the respiration rate remained about the same or even slightly decreased, despite the increased expansion rate of the leaves, which might have been expected to be accompanied by a considerably increased respiration rate. It seems obvious that most of the products of photosynthesis were used in the manufacture of the tissues of the leaf, as reflected in the increased dry weight.

In contrast to the roots of the minus nitrate cultures, those of the plus nitrate cultures gained little in dry weight over the initial cultures. This is in harmony with many findings relative to root-top ratios when plants are liberally supplied with nitrates, and especially if reserve carbohydrates are small in quantity or conditions for their synthesis or translocation limited. It is interesting to note that the volume of the root system of these cultures (table X) increased appreciably and more in relation to root area than did the minus nitrate cultures. This might be expected since the individual roots were much greater in diameter and more succulent than those of the minus nitrate plants. After the application of nitrates the CO_2 output in these experiments rose sharply and increased markedly, such increase being followed presently by an abrupt drop to an amount about equal to tHat of the control plants, and then another slight increase. Since there was some slight increase in the volume of the roots during the experiment, the rate of respiration per unit of volume at the end of the experiment was about equal to that of the initial plants.

Recalling the fact that the roots of the minus nitrate plants, although showing a marked increase in their carbohydrate content, did not show a marked increase in respiration rate, it is safe to say

that the presence of carbohydrates alone in and of themselves do not necessarily mean an increased respiration rate. But if nitrates are added to the nutrient solution, then it is possible that these may become elaborated into amino acids, proteins, and the like, which may have a marked effect on the respiration rate, as SPOEHR's results have previously shown. Thus there could readily be a rapid rise of respiration rate in the case of the roots in these experiments. Similarly such a rise might be expected to be followed by a fall unless the reserve of carbohydrates were very large or unless there was a comparatively large amount of carbohydrates constantly being translocated to the roots. This latter condition apparently did not prevail, such carbohydrates as were synthesized being utilized largely in top expansion. Finally, although nitrates were abundant even after the first brief period, the carbohydrate supply in the roots may well have become a limiting factor in the respiratory rate.

A condition very similar to that shown by these plus nitrate roots was clearly indicated by the tomato leaves, which contained a large supply of carbohydrates at the time of application of the nitrates, and also by the tops in one of the experiments with wheat, when such tops contained a fairly large initial content of carbohydrates. The abrupt rise of respiration rate was not observed in the tops of those plants which did not have such a reserve, even though nitrates were added, as has already been stated.

Comparison of work on tomato and wheat

With respect to respiration, the same general results were secured with both plants. There can be no doubt concerning the resultant marked increase in the respiration rate by the application of nitrate to the plant provided there is an initial carbohydrate reserve. The rate is roughly proportional to the amount of such carbohydrate content. This is true whether the organ experimented upon is leaf or root.

It is equally clear that a relatively high photosynthetic rate may be maintained by leaves low in soluble forms of nitrogen, relatively low in content of chlorophyll, and relatively high in carbohydrate content of the cells. These findings should be accompanied by critical qualitative and quantitative chemical analyses at the time

the demonstrations are made. Detailed analyses are not available for the experiments presented, but roughly quantitative measurements of some of the compounds were made, and have already been commented upon.

The apparatus as herein described is adequate for the determination of CO_2 output and uptake by plants. It is delicate and requires care in its operation, but the results obtained with it under uniform conditions may be relied upon. The next step is to correlate these findings with the chemical composition of the organs being studied and with critical quantitative measurements of areas, volumes, and the like, so that the rates of the processes of photosynthesis and respiration may be precisely determined. This is particularly necessary because the present results, found over and over again in these experiments, are not completely in accord with some previous results as found in the literature and more or less taken for granted as standard and final. The whole subject is still open for further critical experimentation and a revaluation of the data already recorded.

BUREAU OF PLANT INDUSTRY
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LITERATURE CITED

1. BRIGGS, G. E., The characteristics of subnormal photosynthetic activity resulting from deficiency of nutrient salts. Proc. Roy. Soc. B. **94**:20–35. 1923.
2. FRIEDEMANN, T. E., and KENDALL, A. I., The determination of carbon and carbon dioxide. Jour. Biol. Chem. **82**:45–55. 1929.
3. GREGORY, F. G., and RICHARDS, F. J., Physiological studies in plant nutrition. I. The effect of manurial deficiency on the respiration and assimilation rate in barley. Ann. Bot. **43**:119–161. 1929.
4. MITCHELL, JOHN W., A method of measuring respiration and carbon fixation of plants under controlled environmental conditions. BOT. GAZ. **97**:376–387. 1935.
5. ——, Measurement of the area of attached and detached leaves. Science n.s. **83**:334–336. 1936.
6. NIGHTINGALE, G. T., Effects of temperature on metabolism in tomato. BOT. GAZ. **95**:35–58. 1933.
7. SPOEHR, H. A., and McGEE, J. M., Studies in plant respiration and photosynthesis. Carnegie Inst. Washington, Publ. no. 325. 1923.

GRASS SEEDLING ANATOMY: THE FIRST INTERNODE OF AVENA AND TRITICUM

LUCY BOYD[1] AND GEORGE S. AVERY, JR.

(WITH SIX FIGURES)

Introduction

Our knowledge of the anatomy of grass seedlings has developed chiefly through studies of homologies that have been made during the past 100 years. In the last decade a developmental approach has been made in such studies, particularly with reference to the coleoptile of the *Avena* seedling. With the extensive use of this plant for quantitative work in hormone research, it seems especially appropriate at this time to clear up some of the problems connected with the vascular relationships of the cotyledon and coleoptile to the seedling axis. The movement of hormones or their precursors from the cotyledon into the seedling axis, and subsequently into the coleoptile, depends largely upon vascular pathways; similarly, the movement of water from the seedling roots to the cotyledon, coleoptile, and later leaves.

We were prompted to re-examine the vascular relationships of seedling organs of oats and wheat because the epiblast of the seedling in the Gramineae has been interpreted once more as a second cotyledon (7), an interpretation which would necessitate classifying the epiblast-bearing grasses with dicotyledonous plants. There is nothing else about the structure of grasses supporting such a drastic interpretation, or change in their taxonomic position. It is more probable that the grass seedling is in close structural harmony with the seedlings of related monocotyledonous groups.

The external morphology of the embryo and seedling of *Avena* and *Triticum* is so well known that only the briefest reference to it need be made here. The cotyledon consists solely of the "scutellum," and

its vascular system has been described (2). In certain genera such as *Avena* there is an outgrowth present on the face of the scutellum. This is called the "ventral scale," and is a curved ridge which extends downward from above the tip of the coleoptile, partially surrounding the base of the embryonic axis. It is most marked in genera possessing an epiblast. The latter is a parenchymatous, nonvascular structure on the axis opposite the scutellum. The internal root-stem transition occurs about the level at which the epiblast is borne. The ventral scale and epiblast are together interpreted (3) as the ligule of the cotyledon. Such a ligule occurs as a complete structure in the "ligulate type" of monocotyledonous seedling. The level of attachment of the scutellum to the axis marks the first, or cotyledonary node of the plant.

The coleoptile which sheathes the embryonic leaves is interpreted as the first leaf of the plant above the cotyledon, and the level of its attachment to the axis marks the *coleoptilar node*. Structurally the coleoptile is similar in the two genera. It differs in two respects from the later leaves: it is not differentiated into sheath and blade and it possesses only two veins, one on either side. Its simplicity of structure in no way invalidates it as the leaf next above the cotyledon, an interpretation based on its vascular relationship to the axis. Since the coleoptile is the second leaf of the plant (the cotyledon being the first), it follows that the level of coleoptilar divergence from the axis marks the upper limit of the first internode; that is, it is the second node.

The behavior of the cotyledonary bundle upon entering the axis in the seedling is mainly dependent on the location (in the mature embryo) of the meristematic zone which is responsible for the initial elongation of the first internode (2). In *Triticum*, elongation takes place principally in the second internode and is negligible in the first internode. The scutellar trace is therefore relatively unaffected by the actively elongating region of the axis. The corresponding meristematic zone in *Avena* surrounds the scutellar bundle; and when elongation occurs at germination, the scutellar bundle is laid down in an upward direction in the cortex, parallel to the stele.

This study is concerned mainly with the anatomy of the axis between the first (cotyledonary) and second (coleoptilar) nodes; that is,

the first internode and the transition plate at which root structure begins. Further evidence is offered to support the earlier interpretation that this interval is the first internode of the seedling, and thus aid in bringing the seedling of the grasses into morphological harmony with those of other monocotyledons.

Investigation

ANATOMY OF AXIS BETWEEN TRANSITION PLATE[2] AND LEVEL OF COLEOPTILAR DIVERGENCE

Triticum vulgare

The axis between the transition plate and the level of coleoptilar divergence in *Triticum* is exceedingly short (2). In a two-weeks-old seedling the vascular make-up of the stele at the level of coleoptilar divergence (figs. 1, 2) consists of: (1) the two coleoptile bundles, (2) the traces of the next leaf above the coleoptile (a midrib and three pairs of lateral bundles), and (3) the traces of the third leaf (midrib and a pair of lateral bundles). Slight anastomoses at this level do not affect to any extent the stelar anatomy.

The vascular elements of the axis between the transition plate and the coleoptilar node may, for purely descriptive purposes, be resolved into four groups. In no way does this represent the ontogenetic development.

1. A bundle connects the midrib of the second leaf to the transition plate (the nodal plate of the cotyledon). It has no connection with any other leaf trace (fig. 2 *A–E*).

2. The scutellar, coleoptilar, and second leaf traces are connected on the scutellar side of the axis with a "common bundle"; that is, a bundle which passes through the stem for a greater or less distance (fig. 1) but terminates as a leaf trace or traces (5). Each median lateral bundle of the second leaf is united with the neighboring coleoptilar bundle. The bundles which thereby result are joined by the scutellum trace, making a common bundle which extends approximately 0.5 mm. to the nodal plate of the cotyledon, where it is united with the bundle from the midrib of the second leaf. The re-

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[2] The terms transition plate, cotyledonary plate, and nodal plate of the cotyledon are equally useful and interchangeable.

mainder of the system is symmetrical about the plane in which these two bundles lie.

3. A third system involves the remaining two pairs of lateral bundles of the second leaf and the bundles from the third leaf (figs. 1, 2). The midrib of the third leaf and the outer lateral bundles of
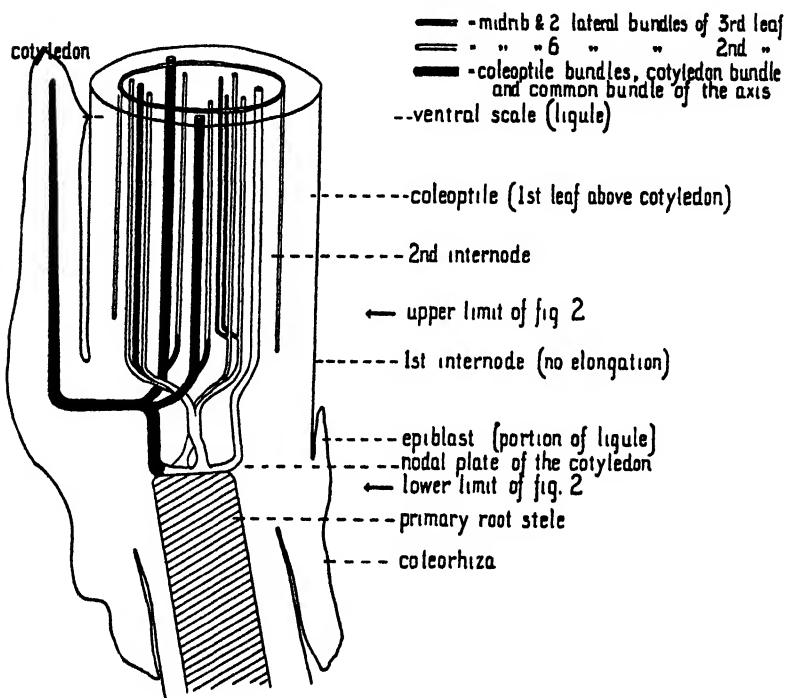
FIG. 1 *Triticum vulgare:* diagrammatic reconstruction of portion of seedling showing principal structures present, and vascular inter-relationships of cotyledon with axis, coleoptile (first leaf above the cotyledon), and first characteristic foliage leaf. Nodal plate of the cotyledon is main level at which root-stem transition takes place. First internode is also in part a transition region. From levels of divergence of cotyledon and coleoptile, it will be understood that the first internode in *Triticum* does not elongate.

the second leaf extend closely parallel. The resulting plexus of these three closely parallel amphivasal bundles connects by two small strands (of half a dozen elements) to the common bundle at the place where it branches to the scutellum and upper leaves (fig. 2 *B*).

- = midrib & 2 lateral bundles of 3rd leaf
○ = „ „ 6 „ „ „ „ 2nd „
● = coleoptile bundles

cotyledon

- - - - - - - - coleoptile
(1st leaf above cotyledon)

- - - - - - - midrib of 2nd leaf
above the cotyledon

- - - level of divergence of coleoptile
„ „ „ „ „ cotyledon

- - - - - - - ventral scale (ligule)

- - - - - endarch collateral bundles

- - - primordia of upper (younger)
pair of seminal roots

- - - - tip of epiblast

- - - - - - primordium of seminal root

- - - - nodal plate of the cotyledon

- - - - - steles of lower (older) pair of
seminal roots

- - - - - stele of primary root

FIG. 2. *Triticum vulgare:* detailed course of vascular bundles and scattered vascular elements in portion of seedling, upper and lower limits of which are indicated in fig. 1.

Each lateral bundle from the third leaf fuses with the neighboring inner lateral bundle of the second leaf (fig. 2 A). The resulting bundles join the plexus just referred to at or slightly below the level where the cotyledonary trace enters the axis (fig. 2 B, C). The vascular network so formed extends through approximately o.3 mm., then divides into two thin plates of tissue, lateral to the two main bundles of the axis; these inclose a small pith (fig. 2 E, F). A little lower down the vascular network consists of the anastomosing tissue of the transition plate (fig. 2 F, G).

4. A pair of roots occurs at the transition plate in a plane parallel to the cotyledon. They are not always at exactly the same level, but the differences are of little importance (fig. 2 D, E). A third root may appear near the epiblast. Since the initials of these roots are present in the embryo, they have been termed seminal roots (8). Like the primary root, each has seven or eight xylem rays in the stele. Where only two arise their relation to the seedling axis is simi-lar. The stele of each seminal root divides; half the elements extend upward, finally uniting with others to form the midrib of the second leaf. The other half forms a trace which bifurcates; one branch joins the inner lateral trace of the second leaf, the other connects with the middle lateral bundle of the second leaf immediately above its fusion with the coleoptilar bundle. The two seminal roots therefore con-nect with the lateral bundles of the second leaf, to a more limited extent with its midrib, and indirectly with the lateral bundles of the third leaf.

A second pair of seminal roots arises a little above but in the same plane as the older set, but for simplification they were omitted in figure 2. This level corresponds roughly with that at which the scutellum trace enters the axis. They connect with the same vascu-lar strands as the·first or lower pair of seminal roots.

Avena sativa

In the young seedling it is this portion of the axis between the cotyledon and the coleoptile which is the principal organ of elonga-tion. It raises the second node and higher nodes (the ultimate aerial portion of the plant) to the surface of the soil. The cotyledonary trace is so located with respect to the meristematic zone that it

differentiates upward in the cortex of the elongating axis (fig. 3). In the mature seedling it appears in a transection of the first internode merely as a cortical bundle.

Just below the level of coleoptilar divergence (fig. 4 *A*) the vascular system of the axis consists of the following: (1) the two coleoptile bundles, (2) the midrib and three pairs of lateral bundles of the second leaf, and (3) the midrib and a pair of lateral bundles of the third leaf. The stelar anatomy is on the same general plan as that of *Triticum vulgare*, and will be described in a similar manner:

1. A bundle extends unchanged from the midrib of the second leaf to the transition plate (figs. 3, 4).

2. Each coleoptilar bundle fuses with the outer and median lateral bundles of the second leaf which lie right and left of it and then joins with the scutellar trace to form a common bundle (figs. 3, 4 *A*). The latter extends downward in the stele parallel to the cortical scutellar bundle. At the transition plate connection is made between the common bundle and the bundle from the midrib of the second leaf (fig. 3).

3. Each inner lateral bundle of the second leaf is united with the neighboring lateral bundle of the third leaf and then with half the midrib of the third leaf (fig. 4 *A*). The resulting bundles extend downward into a meristematic zone (the equivalent level of the "plexus" referred to in *Triticum*), where they lose their identity. It is in this meristematic zone that new cells arise, cells which upon subsequent enlargement are responsible for the elongation of the first internode. In the two-weeks-old seedlings examined, certain highly lignified xylem elements may be traced practically through the meristematic zone. These connect the inner lateral bundles of the second leaf (and associated strands of the third leaf) with elements which extend to the transition plate (fig. 4 *A–E*). The stelar arrangement appears the same in transection from approximately the level of the meristematic zone to the transition plate (figs. 3, 4).

4. A pair of seminal roots is borne at the scutellar node. The vascular connection of these roots is to the elements which lie between the two main endarch bundles (not illustrated, but occurring a little above the level indicated as the nodal plate of the cotyledon in fig. 4 *E*). When three seminal roots occur at this level, the third
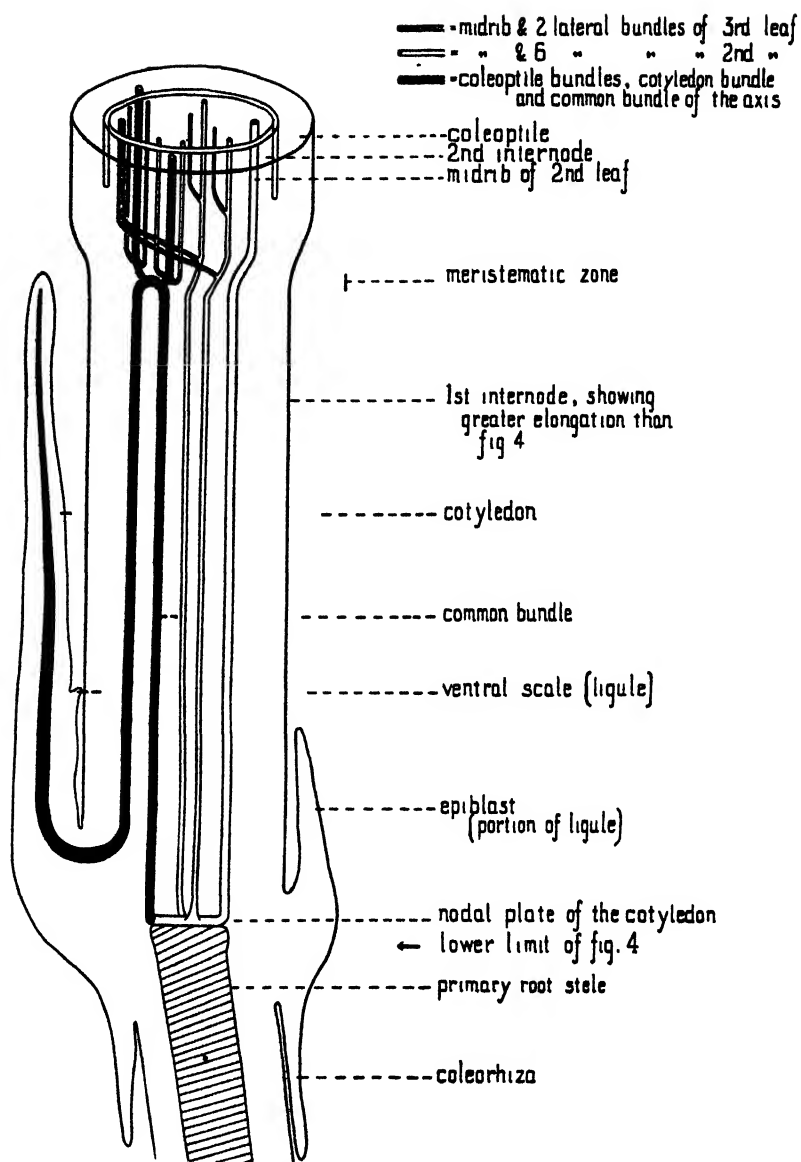
FIG. 3. *Avena sativa*, corresponding to fig. 1 for *Triticum*. First internode has undergone considerable elongation.

Legend labels:

● = midrib & 2 lateral bundles of 3rd leaf
○ = " " 6 " " " 2nd "
⊙ = coleoptile bundles

midrib of 2nd leaf above the cotyledon

coleoptile (1st leaf above cotyledon)

level of divergence of coleoptile

meristematic zone

primordium of adventitious root

tip of ventral scale (ligule)

tip of epiblast

level of divergence of the cotyledon

cotyledon

endarch collateral bundles

nodal plate of the cotyledon

upper portion of transition region

base of ventral scale

FIG. 4. *Avena sativa*, corresponding to fig. 2 for *Triticum* First internode increases in length at its upper end, with new cells arising in meristematic zone indicated, and subsequently elongating.

lies near the epiblast, midway between the other two. In the seedling, two or three adventitious roots appear in the neighborhood of the meristematic zone (fig. 4 B). Less frequently a pair of roots arises in the middle of the internode or near its base. The seminal roots are usually hexarch. Two adventitious roots later emerge at the coleoptilar node. Of the seven xylem strands making up the polyarch stele of the primary root (fig. 4 F), three may be traced to the common bundle, two to the midrib of the second leaf, and one to each of the small interlying strands. The lateral strands (fig. 4 F) connect certain lateral bundles of the second, and the midrib and two lateral bundles of the third leaf with the primary root.

Discussion

The axis between the transition plate and the coleoptilar node is the first internode of seedling grasses. Whether short or long it has an intermediate root-stem structure such as occurs in the first internode of diverse types of seedlings. Its lateral bundles (from the second and third leaves) are neither exarch nor endarch, but the two main bundles are normal endarch collateral bundles (figs. 2, 4).

That we are dealing with an ordinary internode and not a peculiar structure ("mesocotyl," "hypocotyl," "elongated node," etc.) is clear. The cotyledonary bundle branches to supply the coleoptilar traces at the point where it connects with the stele, and then extends downward to the transition plate. The term *cotyledon trace* is reserved for the bundle only to the point where it enters the stele; thereafter it is regarded as a common bundle. The anomaly of the coleoptile drawing its vascular supply directly and entirely from another leaf, the cotyledon, is thereby disposed of. A similar nomenclature would naturally apply to other monocotyledonous seedlings with an identical anatomy in the first internode (figs. 5, 6). That the bundle follows an uninterrupted course from the cotyledon to the transition plate is scarcely an argument against this nomenclature. Similar situations frequently exist at higher levels of the stems of numerous plants.

The normal leaf-axis relationship is obscured in monocotyledonous seedlings by the dominance of the cotyledon. In ontogeny the cotyledonary bundle (or bundles) differentiates while the stem is

still comparatively embryonic. In the ontogeny of higher leaves the main provascular strands similarly differentiate before connection with stem bundles is made.

McCALL (7) has presented detailed observations on the wheat seedling. He interprets that part of the axis between the cotyledonary and coleoptilar nodes, the first internode of AVERY (2) and BOYD (4), as two internodes. The first he places between the attachment of the epiblast and the cotyledon, the second between the
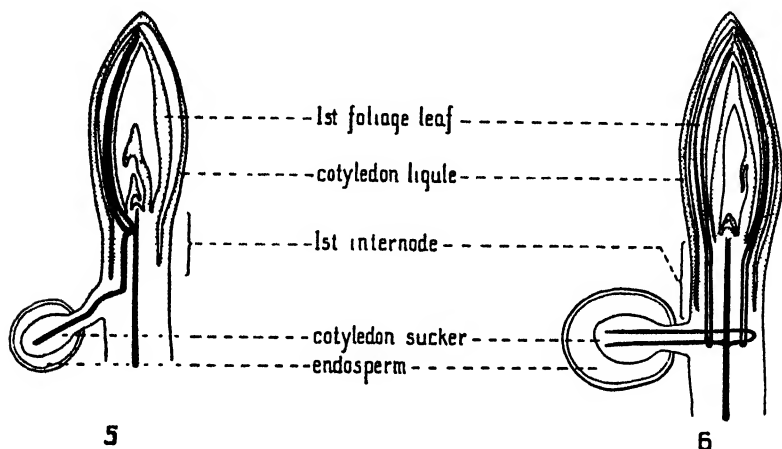
FIGS. 5, 6.—Fig. 5, *Billbergia zebrina:* diagram of two-weeks-old seedling In its main plan the first internode is comparable with that of *Avena* Fig 6, *Alpinia calcarata:* diagram of week-old seedling, illustrating anatomy of first internode, which in its main features is comparable with that of *Triticum*, even though elongating somewhat.

levels of divergence of the cotyledon and the coleoptile; the epiblast is then the "first non-functional leaf," the scutellum the second leaf, and the coleoptile the third.

The vascular plate (fig. 2 *D*) formed by the moving inward of certain traces of the second and third leaves is interpreted by McCALL as the second node. He bases his interpretation on three tenets:

a. *"Vascular connections extend entirely across the central cylinder except for a small pith area."* The occurrence of a vascular plexus does not necessarily imply the existence of a separate node. It is a region of anastomosing lateral traces of the second and third leaves.

An equally plausible interpretation is that it is the upper zone of a transition which seldom requires more than 0.5 mm.

b. "*The scutellar trace diverges from the axis below this plate.*" The question may well be raised as to what constitutes a node. Internal criteria alone are inadequate. According to an early definition (6), a node is "that part of a stem which has a leaf or a whorl of leaves." This interpretation is still in accepted usage. The cotyledonary attachment is more closely associated with the cotyledonary plate than it is with McCALL's plate, and externally its level of attachment extends appreciably above and below it. The plexus may be interpreted as a second node only if the scutellum can definitely be proved to be a second cotyledon; that is, that the epiblast is the first cotyledon. Evidence to support the contention that the epiblast is the first cotyledon would result in classifying the grasses possessing an epiblast with the dicotyledons. Such evidence is lacking. Accordingly, the divergence of the scutellar trace from below McCALL's "plate" does not, in our judgment, provide grounds for his interpretation.

c. "*Adventitious roots are associated with it as in any typical node.*" The presence of adventitious roots does not define a node. Nodal rooting is undoubtedly secondary. A nodal plexus may exist in rootless nodes and adventitious roots may arise in an internode. Comment has been made on the variability in the origin of the second pair of adventitious roots in *Triticum*. It is even more striking in *Avena*.

Certain difficulties arise from McCALL's application of his scheme to *Avena*. The horizontal trace which he labels "second node, scutellum trace divergence" presumably corresponds to the anastomoses of the midrib of the third leaf with two bundles, each comprising lateral traces of the second and third leaves (fig. 4 *A*). It is above the level at which the cotyledonary bundle leaves the stele, and has no direct connection with it. The region in *Avena* most closely approximating the structure of McCALL's second node in *Triticum* is to be found immediately below the meristematic zone in a region of active differentiation (fig. 4 *B*).

Use of the term hypocotyl (7) for the first internode is puzzling.

According to McCall's own interpretation of the *Avena* seedling, he makes hypocotyl and first internode synonymous, and places the two structures which he regards as cotyledons (epiblast and scutellum) at the base of the hypocotyl.

From a study of *Sorghum vulgare*, Reznik (9) notes that the axis between the cotyledonary and coleoptilar nodes shows a resemblance to the hypocotyl rather than to the higher part of the axis, and concludes that the region under discussion has not the structure of an internode but is a special structure, the nodal region of the scutellum. Since hypocotyledonary structure is so variable in different plants, and at different levels in the same plant, comparisons involving it are impossible. His main reasons for regarding the coleoptile as a dependency of the scutellum are those of previous investigators and have already been discussed (2, 3).

It is considered by Arber (1) that the scutellum is equivalent to the blade of the foliage leaf, the coleoptile to its ligule, and the intervening axis designated "mesocotyl" to the cotyledonary node elongated by intercalary growth in its upper region. From our study it appears that the coleoptile bears no closer relationship to the seedling axis or to the scutellum than do the later leaves. A theory which makes the coleoptile part of the cotyledon is of questionable value in view of the occurrence (4) of similar vascular connections in the first internode of seedlings of *Billbergia zebrina* and at least four genera of Zingiberaceae. The first leaf in these seedlings would never be considered part of the normal complete cotyledon which they possess, nor would their first internode be regarded as an anatomical freak. Arber further considers the epiblast and coleorhiza as non-foliar excrescences similar to the "rachilla flap" of certain Gramineae. In those instances with which we are familiar the epiblast and ventral scale consistently occur together and the key to their identity may be found in a study of the seedlings of the Bromeliaceae and Zingiberaceae cited, where the cotyledonary ligule is clearly the equivalent of the ventral scale and epiblast of the grass seedling.

It is difficult to understand the reluctance of students of grass seedling morphology to place the Gramineae in a more natural re-

lationship with the phylum and unfortunate that in this group emphasis frequently has been placed on anatomical differences rather than on likenesses.

Summary

1. A detailed study of the anatomy of the seedling axes of *Triticum vulgare* and *Avena sativa* provides evidence for the following interpretations:

a. The coleoptile is the first leaf above the single cotyledon; its divergence from the axis marks the coleoptilar node.

b. The first internode, whether short (*Triticum*) or long (*Avena*), extends from the cotyledonary to the coleoptilar node. It has intermediate root-stem structure with two main endarch collateral bundles and numerous more or less transitional strands. The old term mesocotyl implies that the first internode is part of the cotyledon; this is not the case, and the term should be dropped from the literature.

c. The hypocotyl which lies between the cotyledonary plate and the upper limit of primary root structure is so short as to be practically negligible.

2. The vascular relationships of seedling organs are discussed in some detail   The evidence from this study does not support recent theories which would make the epiblast-bearing grasses dicotyledonous or the seedling structure of Gramineae an anomaly among monocotyledons.

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· LITERATURE CITED

1. ARBER, AGNES, The Gramineae. Cambridge. 1934.
2. AVERY, G. S , JR., Comparative anatomy and morphology of embryos and seedlings of maize, oats, and wheat. BOT. GAZ. 89:1–39. 1930.
3. BOYD, LUCY, Evolution in the monocotyledonous seedling: A new interpretation of the morphology of the grass embryo. Trans. Bot. Soc. Edinburgh 30:286–302. 1931.

4. ———, Monocotylous seedlings. Morphological studies in the post-seminal development of the embryo. Trans. Bot. Soc. Edinburgh 31:5–224. 1932.

5. EAMES, A. J., and MacDANIELS, L. H., An introduction to plant anatomy. New York. 1925.

6. LINDLEY, J., The elements of botany . . . . and a glossary of technical terms. London. 1849.

7. McCALL, M. A., Developmental anatomy and homologies in wheat. Jour. Agr. Res. 48:283–321. 1934.

8. PERCIVAL, J., The wheat plant. New York. 1922.

9. REZNIK, M. A., Étude anatomique de la plantule de Sorgho. Rev. Gén. Bot. 46:385–419. 1934.

10. SARGANT, ETHEL, and ARBER, AGNES, The comparative morphology of the embryo and seedling in the Gramineae. Ann. Bot. 29:161–222. 1915.

FORMS OF CRATAEGUS PRUINOSA

H. W. RICKETT

(WITH EIGHT FIGURES)

In 1908 and 1911 SARGENT[1] listed 125 species of *Crataegus* from Missouri, most of them described and named by himself. Ten more were added in subsequent years.[2] The collections upon which his species were founded came from 22 counties; six of these form a group in the southwestern corner of the state, seven more are clustered in the southeastern Ozark region, three others are scattered in the Ozarks, three are near St. Louis and one near Kansas City; only four species were described from north of the Missouri River. These species, many of which are known only from the type locality, are associated with such assiduous collectors as *E. J. Palmer*, *J. Kellogg*, and *B. F. Bush* (32 species, for instance, are recorded only from Jasper County, having been collected there mostly by *Palmer*); the absence of records from the remaining four-fifths of the state is due not to the absence of *Crataegus* but to a lack of sufficiently careful collections (as SARGENT himself pointed out[3]). Furthermore, there is little doubt that proper collections would reveal a like profusion of species in many remaining parts of the state; it is evident that many of the *Crataegi* of Boone County, for instance, cannot be identified with any of SARGENT's species.

The current aspect of the "*Crataegus* problem" almost justifies the prediction of ADANSON about the future of species,[4] and the attitude even of many taxonomists toward it is one of hopelessness. The description of additional forms would seem to be merely reckless. If, however, we are to understand any local flora, it is necessary that the distinguishable entities in this genus be collected and described and some provision made for a rational and usable nomenclature. For

[1] Rep. Mo. Bot. Gard. **19**:35–126. 1908; **22**:67–83. 1911.

[2] Trees and shrubs **2**:236–245. 1913; Jour. Am. Arb. **1**:253. 1920; *ibid.* **3**:2–10, 82. 1922; *ibid.* **6**:2–4. 1925.

[3] Rep. Mo. Bot. Gard. **22**:67. 1911.

[4] Fam. Plantes; cited by BRITTON in Amer. Nat. **42**:226. 1908.

several years it has been one of my cares to attempt to learn something of the *Crataegi* of the vicinity of Columbia, Missouri. The present paper is limited to the few Pruinosae of this region, but treats these in a way which perhaps may contribute to the elucidation of the general problem.

It was long ago pointed out by such students as BRITTON[5] and BEADLE[6] that for a proper understanding of the species of *Crataegus* it is necessary to observe the plants in the living condition and at various times in the year. In order to study also the more minute characters it is desirable to collect from the same trees during successive years. A number of plants near Columbia have been marked with numbers; 20 of these being Pruinosae. These trees (with, of course, many others which were not labeled) I have had under close observation during three years (1931–1933). During parts of these years when I was absent I have had the valued assistance of Dr. FRANCIS DROUET. In the spring of 1934 collections and observations were made by Miss ROSE KENTNER. It is a pleasure to acknowledge my indebtedness to these helpers.

In *Crataegus*, as in other polymorphic genera, the few broadly conceived species of earlier workers have been replaced by a multitude of more homogeneous groups, often distinguishable only by minute characters; of such a nature are, for instance, the species described by SARGENT. Such groups have been termed elementary species or microspecies. The chief problem in the study of *Crataegus* is the taxonomic treatment of these groups; without prejudicing the conclusion finally reached, I shall refer to them rather loosely during the ensuing discussion as forms or types.

Three types of Pruinosae may be distinguished in the neighborhood of Columbia. They differ in a number of characters, such as color of anthers, number of stamens and of styles, margination of sepals, pubescence of leaves ·and of inflorescence, length of spines. The characters in themselves are unimportant and often difficult to detect, but the correlation between them is striking and interesting.

The most obvious differences are in the color of the anthers, these being red, rose-pink, or cream-white. The number of stamens in all

⁵ Bot. Gaz. 22:222. 1896.   ⁶ Bull. Torr. Bot. Club 37:255. 1910.

plants seen is about 20; in view of the variations in number even among the flowers of one plant, this statement would suffice for ordinary systematic purposes. When, however, actual counts are treated statistically, significant differences appear between plants characterized by anthers of different colors. For this purpose, the stamens were counted in ten flowers of each plant, the mean and standard deviation being calculated for each plant so studied. The results are presented in table I. It will be noted that with three exceptions (numbers 2, 5, 17) plants alike in color of anthers are remarkably uniform in the mean number of stamens per flower, and these numbers are significantly different in two of the groups. Of the exceptions, two are exceptional only in one year; this makes it probable that the statistical methods used were not entirely adequate to deal with such a fluctuating character, and that the other exception may be similarly explained, particularly since this plant belongs to the group with pink anthers, in which the number of stamens seems to be regularly more variable. The number of styles was studied in the same way (the same flowers furnishing both series of figures); exactly the same statements may be made about the results.

The length of spines is an extremely variable and unsatisfactory character; but, as may be seen from the table, random measurements (made on herbarium specimens) of the spines mostly of young flowering or fruiting shoots show an obvious difference between two of the groups. This confirms the impression gained from field observation.

The sepals of all the plants are more or less glandular on the margins. Those of plants which bear white anthers, however, are very sparingly so, many of them being entire save for a few delicate cilia. Sepals of flowers which contain pink anthers are regularly provided with long-stalked glands on their middle portions. In spite of the apparent variability of this character, so close is the correlation between the nature of the margin of the sepals and the color of the anthers that it is possible to predict with confidence the color of the anthers in unopened flowers by an examination of a few buds; almost never does one make a mistake. The differences are illustrated in figures 1–8.

TABLE I

PLANT NO.	MEAN NUMBER OF STAMENS PER FLOWER	MEAN NUMBER OF STYLES PER FLOWER	LENGTH OF SPINES (MM.)
	Plants with white anthers		
2*	17.7 ±0.40	4.3 ±0.17	38.5 ±0.29
	19.6 ±0.27	4.9 ±0.06	
4	20.0 ±0.09	4.7 ±0.10	40.5 ±0.24
	19.3 ±0.26	4.5 ±0.10	
7	19.8 ±0.16	4.0 ±0.13	42.2 ±0.25
	19.3 ±0.25	4.4 ±0.10	
9			44.7 ±0.25
12			40.3 ±0.31
17	17.6 ±0.37	4.0 ±0.16	
	19.7 ±0.21	4.4 ±0.17	
18	19.4 ±0.19	4.2 ±0.13	38.1 ±0.20
Unlabeled plants	18.6 ±0.27	3.6 ±0.10	
	19.2 ±0.16	4.3 ±0 13	
	19.8 ±0.15	4.5 ±0.11	
	19.3 ±0.28	4.2 ±0.15	
	19 8 ±0.31	4.3 ±0.13	
	19.5 ±0.19	4.6 ±0.10	
	19.8 ±0.16	4.5 ±0.14	
	19.9 ±0.06	4.3 ±0.17	
	19.4 ±0.25	4.7 ±0.10	
	19.5 ±0.15	4.4 ±0.11	
	Plants with pink anthers		
3	17 4 ±0.35	3.4 ±0.10	25.7 ±0.22
5	19.2 ±0.31	3.8 ±0.16	26.7 ±0.21
	20 0 ±0.16	4.2 ±0.13	
15	18.6 ±0.27	3 7 ±0.18	
	17.4 ±0.33	3.0 ±0.19	
26			23.5 ±0.12
27			25.7 ±0.11
32			36.7 ±0.22
61	18.6 ±0.28	2.9 ±0.11	
Unlabeled plants	18.9 ±0.29	3.3 ±0.16	
	17.9 ±0.36	3.4 ±0.14	
	17.0 ±0.33	3.5 ±0.17	
	17.2 ±0.40	3.2 ±0.19	
	18.3 ±0.40	3.5 ±0.14	
	18 9 ±0.22	3.8 ±0.16	
	17.6 ±0.24	3.7 ±0.14	
	18.1 ±0.22	3.2 ±0.13	
	18.8 ±0.33	3.4 ±0.11	
	18.9 ±0.20	3.4 ±0.14	

* Two entries for one plant represent counts made in successive years.

TABLE I—*Continued*

PLANT NO.	MEAN NUMBER OF STAMENS PER FLOWER	MEAN NUMBER OF STYLES PER FLOWER	LENGTH OF SPINES (MM)
	Plants with red anthers		
14	19 8 ±0 13	4 2 ±0 18	37 3 ±0 02
	19 5 ±0 14	4 2 ±0 18	
Unlabeled	19 7 ±0 13	4 3 ±0 10	
plants	19 8 ±0 08	4 2 ±0 13	
	19 5 ±0 17	3 9 ±0 18	

Almost all the plants are nearly or entirely glabrous. In this respect also, however, close examination reveals constant differences. The plants with red anthers are distinguishable from those with pink anthers by the presence on the upper surfaces of their leaves of a thin glistening pubescence; in maturity they may be slightly scabrid. The inflorescence also is sparingly pubescent. The plants with pink anthers are entirely glabrous from the first. Those with white anthers are sometimes glabrous; but frequently at least some of the young leaves bear on their upper surfaces a few short glistening hairs in the areas between the principal veins.

The types may be distinguished also by the size and number of the nutlets, and by other characters of fruits. I have not as yet satisfactory evidence of the uniformity of these characters.

The constancy of the preceding characters during the period of observation has been remarkable. The plants grow in several widely separated situations, mostly rocky, well drained hillsides (where they are mingled with other species of *Crataegus*). The forms characterized by pink and by white anthers grow together in all of the localities where the Pruinosae are abundant, and specimens of either type collected from different localities resemble each other as closely as do those from the same spot. The more pubescent form with red anthers has been found in only one place, where it is associated with the two others and with other species. Particular attention was given to trees growing in close association, in order to detect any signs of intergradation between the different types; the results were entirely

negative, plants so close that their branches were interlaced, and blooming at the same time, being easily separable into the groups just described.

It is not, of course, proposed that systematists should be prepared
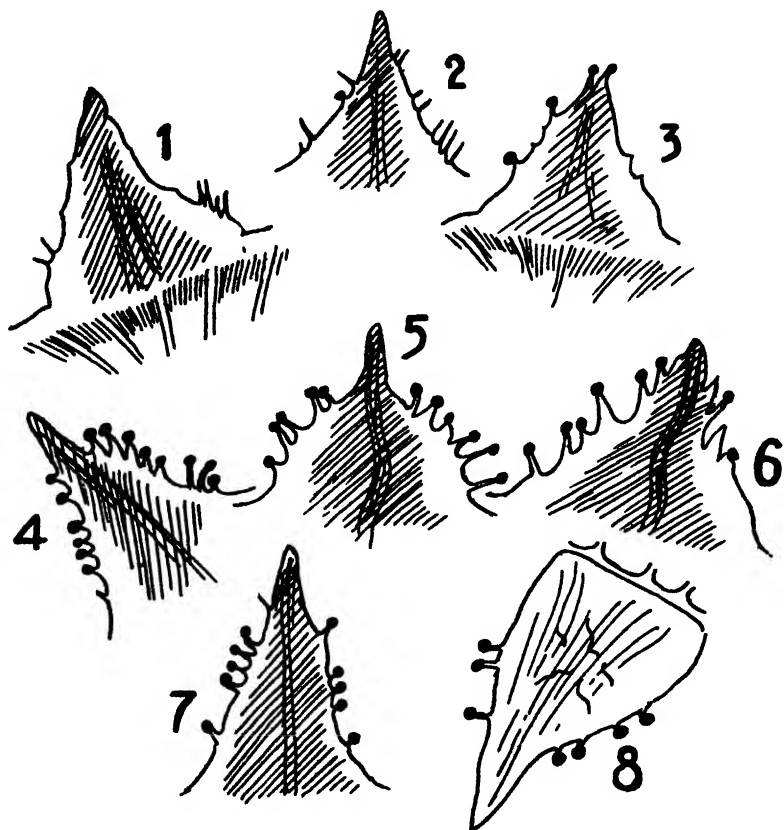
FIGS. 1-8.—Sepals of Pruinosae: figs. 1, 2, 3, plants with white anthers; figs. 4, 5, 6, plants with pink anthers; figs. 7, 8, plants with red anthers. ×5.

to make statistical calculations of the numbers of stamens or of styles before being able to determine the name of a species, variety, or form; nor that the characters which have been used in this study are necessarily of value in other sections of the genus.[7] But the existence

[7] PALMER writes that the glands of the sepals, for instance, are so variable in some species as to be of small taxonomic value.

of such types and the possibility of such distinctions as those described in this paper warrant certain conclusions, which may be of general value.

The cultivation of *Crataegus* at the Arnold Arboretum has demonstrated the remarkable constancy of species when grown from seed.[8] Many of the species, nevertheless, including *C. pruinosa*, are thought to be of hybrid origin. This is based on their polyploid chromosome numbers and on the large proportion of sterile pollen grains associated with irregularities in meiosis.[9] Polyploidy is often taken as indicating hybridity, although of course it does not necessarily do so.[10] In any case, the existence of constant lines within a species marked by both polyploidy and pollen sterility makes it probable that the reproduction is apomictic.[11] The close correlation of a number of small characters in each of the three types described in this paper, and the entire absence of any individuals which show the same characters in different combinations, also indicate that each type propagates itself without interbreeding and probably apomictically. Experiments by SAX, reported by PALMER (*l.c.*: 357), have shown that castrated flowers of a number of species, including *C. pruinosa*, set fruit at least sometimes.

The connection of polyploidy, hybridity, and polymorphism with abnormalities in reproduction is well known.[12] The method of reproduction cannot, of course, be inferred from a knowledge of the chro-

[8] PALMER in Jour. Arn. Arb. **13**:350. 1932; BROWN in Bull. Torr. Bot. Club **37**: 254–255. 1910.

[9] LONGLEY (Amer. Jour. Bot. **11**:300. 1924) reports *C. pruinosa* a triploid with "24 diploid chromosomes." He calls species with 16 bivalents diploid and those with "32 gametophytic chromosomes" tetraploid.. MOFFETT (Proc. Roy. Soc. Lond. B **108**:438; fig. 50. 1931) lists the same species as having a reduced number of 34, and calls it tetraploid (not diploid, as PALMER says, *l.c.* :357), the basic number of the genus being, as in other Pomoideae, 17. ʻIt is possible that different forms of this and other species are characterized by different chromosome numbers (see also SAX in Jour. Arn. Arb. **12**:7. 1931). For pollen sterility in *Crataegus* see LONGLEY, *l.c.*: 313. 1924; STANDISH in Jour. Hered. **7**:266–278. 1916; SAX, *l.c.*, figs. 1, 6, 7, 8.

[10] See, for instance, JEFFREY, LONGLEY, and PENLAND in Sci. **55**:517–518. 1922; and compare the views of SAX (Jour. Arn. Arb. **13**:363–367. 1932) and MOFFETT (*l.c.*: 423–446) on the origin of the chromosome number 17 in *Malus*.

[11] *Cf.* SAX in Jour. Arn. Arb. **12**:18. 1931.

[12] BLACKBURN and HARRISON in Ann. Bot. **35**:184. 1921; WOODWORTH in Jour. Arn. Arb. **12**:214. 1931.

mosomal abnormality. In *Malus theifera* according to SAX,[13] and in species of *Rosa* according to HURST,[14] the course of meiosis is entirely different in megaspore and microspore mother cells; most of our knowledge of polyploidy in *Crataegus* and other genera is based almost entirely upon a study of pollen and is strikingly silent on events in the ovule. Castration experiments also may give little information upon the origin of the embryo. In *Zygopetalum*, according to SUESSENGUTH,[15] although pollination is necessary to the formation of the embryo, the pollen tubes do not penetrate the ovules and the embryos arise apomictically. In *Allium odorum*, as described by MODILEWSKI,[16] parthenogenetic embryos arise from diploid cells of the embryo sac only if pollination has occurred and one of the male nuclei has united with the polar nuclei. Similar phenomena of "pseudogamy" are indicated by experiments with *Rubus*[17] and *Potentilla*.[18] The cross pollinations reported by BROWN (*l.c.*, 259) give consequently little information on the actual source of the embryos of *Crataegus*. They may perhaps be formed in a number of different ways even in one species or variety, and these methods may include both apomixis and occasional normal syngamy, as in *Allium odorum* (MODILEWSKI, *l.c.*).

The constancy of the different types might result, as PALMER (*l.c.*: 356) suggests, from physiological differences which prevent interbreeding; effective cross pollination might fail through slight differences in the dates of shedding of pollen and of receptivity of stigma. It is true that the white-anthered type here described blooms slightly earlier than the others; but the flowers of one plant do not open all at once, and the flowering periods of the different types overlap. Even though such conditions may reduce the likelihood of hybridization in otherwise normally amphimictic races, the existence of bad pollen and of polyploidy, with such a high degree of constancy, makes apomixis almost certainly the usual method of reproduction.

[13] Jour. Am. Arb. **13**:365. 1932.
[14] Zeit. Ind. Abst. Vererb. suppl. **2**:891 ff. 1928.
[15] Ber. Deutsch. Bot. Ges. **41**:16–23. 1923.
[16] Ber. Deutsch. Bot. Ges. **48**:285–294. 1930.
[17] LIDFORSS in Zeit. Ind. Abst. Vererb. **12**:1–13. 1914.
[18] MÜNTZING in Hered. **11**:267–283. 1928.

The existence of apomictic lines is not, of course, incompatible with their origin through hybridization. Such a situation is known or suspected in other polymorphic genera, such as *Hieracium*,[19] *Rosa*,[20] *Rubus*,[21] and *Betula* (WOODWORTH, *l.c.*).

When we turn from the description of races to the problem of naming them, the troubles of the taxonomist begin. The application of the traditional species concept to the great polymorphic genera seems to have become very difficult if not impossible. One source of trouble is that the concept of species is, as has been often pointed out,[22] descended from pre-evolutionary times. The Linnean concept of a species (as has been pointed out by BLAKE,[23] by RAUNKIAER,[24] and by CHASE[25]) was not a broader concept than ours; it was not intended to embrace an aggregate of subordinate units, but was a homogeneous group maintaining itself through reproduction. (The varieties of LINNÉ and his contemporaries were ascribed to environmental differences, or to hybridization.[26]) With the development of modern technique the distinguishable units have become smaller and smaller,—the so-called microspecies; and with the recognition that species may be still in the making interest has become centered upon groups of approximately uniform genic constitution,—the biotypes (genotypes) of JOHANNSEN or the isoreagents of RAUNKIAER (*l.c.*: 230, 236). Such are the races of *Crataegus* described in this paper and probably most of the species which have been described in this genus. Meanwhile the idea of the species, with its binomial burden, has striven desperately to adapt itself to the new outlook. On the one hand, species names have threatened to become so multitudinous as to be useless, on the other so broad as to be meaningless; in no case can they fulfill the original definition of a species.

The limits and homogeneity of species have varied with the assiduity and interests of the botanists concerned, so that there is some

[19] OSTENFELD in Zeit. Ind. Abst. Vererb. 3:241–285. 1910.

[20] HURST, BLACKBURN, and HARRISON, *l.c.*

[21] LONGLEY in Amer. Jour. Bot. 11:249–282. 1924.

[22] BAILEY in Bot. Gaz. 22:454. 1896 and Proc. Int. Cong. Plant Sci. 2:1428. 1929; HARPER in Amer. Jour. Bot. 10:229–233. 1923.

[23] Rhod. 20:22. 1918.   [25] Amer. Jour. Bot. 8:48. 1921.

[24] Zeit. Ind. Abst. Vererb. 19:226. 1918.   [26] Phil. Bot. 100. 1751; Gen. Plant. 1764.

justification for the common point of view expressed by BAILEY,[27] GRAY,[28] SHULL,[29] and many others, that the concept of species is mainly a matter of convenience. On the other hand the system of LINNÉ would not have been possible had not his species corresponded to something in nature. Although the Linnean concept is no longer valid, many of the groups of plants which LINNÉ named as species are still recognized as such. In short, the microspecies seem to group themselves into larger assemblages, just as the latter group themselves into genera. As MÜNTZING, TEDIN, and TURESSON state: "We are free to discuss which of these units should be called species, since we do not know 'Nature's own opinion on this matter.'"[30]

It is becoming generally recognized that to treat as species the vast number of recognizable types found in such genera as *Crataegus* is neither practicable nor desirable.[31] Strangely enough, to consider such types species savors strongly, as COWLES pointed out,[32] of a return to a creationist point of view—the belief in fixed units. Actually the Linnean concept is no more applicable to them than to anything else in nature, for even these smaller groups are not necessarily true breeding nor constant, and a nomenclature which recognized them explicitly as such would become more and more chaotic with the passage of time. In spite of the success of genetics, the methods of that science, as BRITTON[33] and RAUNKIAER (*l.c.*, 233–235) have shown, cannot replace the methods of taxonomy in describing the units of classification; species and their subdivisions must continue to be founded on the similarities observed in nature, not on pedigrees nor chromosomes. If we are to have something to call a species, it must be something rather constant.

Among the many recent attempts to devise a system for the naming of subspecific units, the work of TURESSON is well known.[34] He

[27] Bot. Gaz. 22:457. 1896.

[28] Amer. Jour. Bot. 10:222. 1923.   [29] Darwiniana 35. 1889.

[30] Hered. 15:8. 1931; *cf.* remarks of DE WILDEMAN in Proc. Int. Cong. Plant Sci. 2:1413–1421; and of WIEGAND, *ibid.*: 1575. 1926.

[31] The classic defense of "splitting" is RYDBERG'S paper in Proc. Int. Cong. Plant Sci. 2:1539–1551. 1926.

[32] Amer. Nat. 42:268. 1908.   [33] Amer. Nat. 42:238. 1908.

[34] Hered. 3:100–113, 211–350. 1922; 6:147–236. 1925.

has shown that differences among the plants of a single species which
have usually been ascribed to environmental factors often represent
"ecotypes" of different hereditary constitutions. The ecotypes corre-
spond roughly to the varieties of conventional taxonomy, and their
naming presents no serious difficulty (whether old names be pre-
served or TURESSON's suggestions be followed). These groups are
not, however, comparable to the microspecies of the large polymor-
phic genera; each may be a group of different biotypes, often capable
of free interbreeding, the entire complex being held constant largely
by the selective action of the environment. The taxonomic problem
is more acute in such genera as *Viola* and *Rosa*, in which a single
broadly conceived species may consist of an almost indefinite num-
ber of interbreeding races, spread over a wide territory, and all en-
titled to some taxonomic recognition. Any maker of names may well
pause before the more than five million such forms mentioned by
CLAUSEN[35] in the collective species *Viola tricolor*. There is much to
be said for a system of numbers for the subordinate units of such
species, as was long ago suggested by BRITTON.[36] CLAUSEN used
symbols for the forms of *Viola* which he studied; more recently
ERLANSON[37] has treated various forms of *Rosa* by means of numbers
and letters. Certain species (called agamospecies by TURESSON[38])
differ from those just mentioned in that their component races are
apomictic, and therefore constant. An example described by TURES-
SON is *Festuca ovina*, which in certain regions consists of "viviparous"
races.[39] A similar situation exists in certain sections of *Hieracium*
(OSTENFELD, *l.c.*). *Crataegus pruinosa* and perhaps other species (*sen-
su lato*) of *Crataegus* seem to be of this type.

While the number of races of *Crataegus pruinosa* may be very large
(particularly since, as was pointed out at the beginning of this paper,
there is no reason to think that even in this one state all have been
collected and described), the situation is perhaps not so dishearten-
ing as in some species of *Viola* and *Rosa;* because of the method of
reproduction, and for other reasons, single races are not widespread
and the number found in any one locality is not very large. I ven-

35 Bot. Tids. 37:392. 1922.
36 Amer. Nat. 42:241–242. 1908.
37 Bot. Gaz. 96:197–259. 1934.
38 Hered. 12:323–334. 1929.
39 Hered. 8:160–206. 1926.

ture, therefore, to append formal names and descriptions of the types described in this paper. They will be useful only to students of a limited portion of Missouri, and among them only those willing to devote careful observation to the plants; however, if a similar disposition is made of the forms peculiar to other localities, the collective species as a whole will be the better known.

A difficulty with present nomenclature is the lack of definition of the subspecific categories which are prescribed. In describing the races of *Crataegus pruinosa* as forms, I am following what seems to be general practice;[40] such forms also correspond to the *forma apomicta* of TURESSON.[41] Some botanists, however, such as CLAUSEN (*l.c.* 396), designate as forms merely environmental modifications. It is probable that methods of naming should differ in different genera. Whatever be the designation of subordinate races, it should be recognized that none of them is to be considered the "true species" from which the others diverge (except, of course, for nomenclatural typification). If species are to be broadly conceived as composed of many distinguishable groups, some sort of trinomial system must come into general use; agreement on the methods to be used may well be a sign that taxonomic nomenclature has definitely outgrown its heritage of pre-evolutionary thought.[42]

A survey of all the Pruinosae may show that it is possible to distinguish among them several large species, in which the multitude of forms may be classified. Failing clear morphological distinction, it may perhaps be feasible to create geographical species, purely for convenience in listing the forms, which seem to be largely local. Whatever method of treatment finally emerges, it should give proper recognition to the hundreds of microspecies described by SARGENT and others; they cannot be retained as species, but they represent nevertheless real groups.[43]

[40] See WIEGAND, *l.c.*: 1575. *Cf.* COCKERELL's note in Torr. **34**:42. 1934.

[41] Hered. **8**:160–206. 1926.

[42] The Linnean concept is still represented by such recommendations as those of LAUJOUW in Fedde Repert. Sp. Nov. Beih. **66**:200. 1932. He argues that varieties are divergents from a true specific type.

[43] Through the kindness of the staff of the Herbarium of the Missouri Botanical Garden I have been able to examine collections from the type localities, often from the

It may be impossible to identify one of the Pruinosae as *forma genuina*, since no type specimen of *C. pruinosa* is known, and the original description [44] is naturally not sufficiently detailed to permit of distinction from the multitude of known forms. KOCH, who transferred the species to *Crataegus*,[45] left specimens now in the Herbarium of the Botanic Garden at Berlin.[46] One from ENGELMANN, collected near St. Louis, labeled in KOCH's hand, has about 20 stamens, from 4 to 5 styles, strongly glandular sepals, is somewhat pubescent, and has cordate leaves. Another sheet is labeled (also by KOCH) "β *icosandra* (*chlorocarpa*)." It has sepals nearly entire, leaves more tapering at the base. Neither of these fits WENDLAND's description in the characters which distinguish the forms of the species. They serve merely to show that the specimens grown in Europe from which the species was named already included several forms.

Specimens of the following new forms are deposited in the Herbarium of the University of Missouri.

CRATAEGUS PRUINOSA (Wendl.) Koch **ciliata** f. nov.

Frutex gracilis; folia basi cuneata, fere glabra interdum supra paucis setis brevibus praedita; ramuli purpurei, glauci, post castanei tandemque cinerei; spinae similes, circiter 40 mm. longae, rectae vel leviter curvae; inflorescentia glabra, 3–5 floribus; sepala triangulato-acuminata, circa 4 mm. longa, marginibus scariosis saepe integribus vel leviter glandulosis et paucis ciliis exiguis praeditis; antherae eburnae; stamina circiter 19 (15–22); styli circiter 4.5 (3–5); fructus rubri, pruinosi, depresso-globosi, 10–15 mm. lati; nuculae 4–5, angulatae vel late costatae, 6 mm. longae.

Rickett, May 3, 1931 (Herb. Univ. Missouri 8888); *Drouet*, Oct. 4, 1931 (Herb. Univ. Missouri 9535).

type trees, of most of SARGENT's Missouri Pruinosae. SARGENT's descriptions, also, are sufficiently detailed so that one can easily distinguish them from each other and from the forms described in this paper.

[44] *Mespilus pruinosa* Wendl. in Flora 6:699–701. 1823.

[45] Hort. Dendr.: 168. 1853.

[46] I am indebted to DIELS for the privilege of examining these specimens.

CRATAEGUS PRUINOSA (Wendl.) Koch **glaberrima** f. nov.

Frutex gracilis; folia basi cuneata vel truncata, glabra; ramuli virides vel purpurei, glauci, post aurantiaci tandemque cinerei; spinae similes, circiter 30 mm. longae, leviter curvae; inflorescentia glabra, 3–6 floribus; sepala triangulato-acuminata, circa 4 mm. longa, marginibus scariosis glandulosis incisis; antherae roseae; stamina circiter 18 (14–22); styli circiter 3.5 (2–5); fructus rubri saepe viridi-maculati, pruinosi, depresso-globosi, 10–15 mm. lati; nuculae 3–5, saepe late canaliculatae, 7 mm. longae.

Rickett, May 3, 1931 (Herb. Univ. Missouri 8890); *Drouet*, Oct. 4, 1931 (Herb. Univ. Missouri 9558).

CRATAEGUS PRUINOSA (Wendl.) Koch **puberula** f. nov.

Frutex vel arbor compacta; folia basi truncata vel subcordata, supra parce pubescentia setis brevibus nitidis, subtus glabra; petioli pubescentes; ramuli virides vel purpurei, glauci, post fulvi tandemque cinerei; spinae purpureae, glaucae, post atro-rubrae nitentesque, tandem canae, circiter 35 mm. longae; inflorescentia parce pubescens, 3–5 floribus; sepala lanceolata, circa 5 mm. longa, parce glanduloso-serrata; antherae rubrae; stamina circiter 19.5 (18–21); styli circiter 4 (3-5); fructus virides vel rubescentes, pruinosi, depresso-globosi, 10-20 mm. lati; nuculae 4–5, canaliculatae, 6 mm. longae.

Rickett, May 5, 1931 (Herb. Univ. Missouri 8901); *Drouet*, Oct. 8, 1931 (Herb. Univ. Missouri 9544).

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PHOSPHORUS RELATIONS OF LEMON CUTTINGS GROWN IN SOLUTION CULTURES

A. R. C. HAAS

A study was made of the concentration of phosphate in the culture solution in relation to the growth and composition of lemon cuttings. Various concentrations of phosphate in the culture solution affect the absorption of other elements, and when an element becomes a limiting factor, the accumulation of high concentrations of other nutrient elements follows.

Rooted Lisbon lemon cuttings were grown in an unaerated culture solution lacking phosphate. This solution is that described by HAAS (5) modified by the substitution of potassium nitrate for potassium acid phosphate. Different concentrations of phosphate were added as potassium acid phosphate. The cultures were grown in $8\frac{1}{2}$ quart capacity Swedish enamelware pans. In some cases pans of 21 quart capacity were used for the low phosphate concentrations, but without affecting the growth.

It was desirable to sample the culture solutions from time to time in order to ascertain whether the phosphate concentration in the solution was being maintained. At first this was attempted as a guide for removal of the culture solution, but the facilities at hand did not allow its continuance.

In the preliminary experiments the solutions were renewed every other day. For a time the solutions of the duplicate set of cultures were renewed every day, with no resulting improvement. Sharp contrasts of growth within a relatively narrow range of phosphate concentration were not found, and consequently the cultures were grouped into somewhat wide ranges of phosphate concentration. It was desirable to maintain a given phosphate concentration in the solution continuously, yet this was possible in theory only, since the lemon cuttings require iron as well as phosphorus. When iron and phosphorus are both present at the same time in the culture solution,

varying amounts of both are precipitated. In practice, therefore, it
was found best to maintain the phosphate for a time and then re-
move it; another method used was to allow the phosphate to remain
while an abundant supply of iron was added.

The first set of cultures was conducted in duplicate, and the solu-
tion in one group contained a trace of aluminum while the solution
in both groups contained ferric tartrate. The aluminum and iron were
precipitated by the phosphate, which greatly altered the concentra-
tion of soluble phosphate. The concentrations of phosphate added
to the culture solution were 0, 0.0125, 0.0250, 0.050, 0.075, 0.1, 0.2,

TABLE I

PHOSPHORUS AS A PERCENTAGE OF DRY MATTER
OF LISBON LEMON CUTTINGS*

PO₄ IN CULTURE SOLUTION (P.P.M.)	ORIGINAL LEAVES	NEW LEAVES	ROOTS
0.	0.0593	0.0556	0.0870
0.0125.	0.0517	. . . .	. . . .
0.05.	0.0508	0.0820	. . . .
0.10.	0.0467	0.0674	. . . .
0.20.	0.0545	0.0641	0.1192
105.	0 0847	0.1879	. . . .

* Cuttings grown from January 26 to July 9, 1934, in solution cultures
presumed to contain certain low concentrations of phosphate.

and 105 p.p.m. The percentage of phosphorus in the various por-
tions of a number of the cuttings was determined at the conclusion
of the experiment. The data are shown in table I.

The results give little information other than that at the highest
phosphate concentration the original leaves of the cuttings contain
a fairly high percentage of phosphorus. In such studies it is essential
to keep iron out of the solution during the intervals in which the
phosphate concentration is being maintained. Studies of phosphate
nutrition involve, therefore, a consideration of the iron nutrition.
The growth and composition of citrus cuttings will differ, not only
according to the composition of the culture solution, even as regards
certain traces of elements, but also according to the technique em-
ployed in using these solutions. Environmental variations add fur-
ther to the complexity of the results.

Another set of Lisbon lemon cultures run in duplicate was begun on July 3 and concluded on December 19, 1934. The solutions were not aerated but were changed every few days and were given iron a few hours prior to such changes   In order to simplify the labor in-

FIGS 1, 2 —Lisbon lemon cuttings grown from July 3 to December 19, 1934, in un-aerated solution cultures containing various concentrations of phosphate Left to right fig 1, 0, 0 0125, and 0 025 p p m , fig 2, 0 050, 0 075, 0 1, and 0 2 p p m

volved, no aluminum was used in these cultures. The following con-centrations of phosphate as potassium acid phosphate were added to a culture solution lacking phosphate: 0, 0.0125, 0.025, 0.050, 0.075, 0.1, 0.2, 0.3, 0.5, 1 0, 2.0, 5.0, 7.5, 10 5, 15.75, 21.0, 26.25, 31.0, and 105 p p m. To secure a relative increase in the volume of solution at the lowest concentrations, the cultures each contained only two cuttings, while at higher concentrations the number was increased to three. The cuttings were photographed and then were grouped into

certain ranges of phosphate concentration. The growth obtained for each of these ranges is shown in figures 1-4.

Figures 1 and 2 illustrate the growth of cuttings in solution cultures containing from 0 to 0.2 p.p.m. phosphate. Only two cuttings

FIGS 3, 4 —Same as figs 1, 2 Left to right fig 3, 0 3, 0 5, 1, and 2 p p m , fig 4, 5, 7 5, and 10 5 p p m , cuttings with the best root systems occurred in the cultures containing 1 to 2 p p m phosphate

were used in each of the 8½ quart capacity pans. None of the plants could be said to be free of symptoms of phosphorus deficiency, regardless of how frequently it was possible to renew the solutions. No concentration of phosphate within this range proved superior to the others for the growth of the cuttings employed.

Figures 3 and 4 show the growth typical of duplicate cultures of Lisbon lemon cuttings produced in solutions containing from 0.3 to 10.5 p.p.m. phosphate. Three cuttings were used in each culture. The healthiest appearing root systems in conjunction with a top that just has sufficient phosphorus are seen to occur in figure 3 at roughly 1 to 2 p.p.m. Very slight symptoms of phosphorus deficiency occurred in the external appearance of young leaves from cuttings grown in a culture solution containing 1 p.p.m. phosphate but none at 2 p.p.m. At 5 p.p.m. and higher, the rootlets became more finely divided and less healthy, the cause for which will be pointed out later.

A photograph was made of the growth obtained when the duplicate cultures, each containing three plants, were grown in a solution containing from 15.75 to 105 p.p.m. of phosphate. The growth produced was not appreciably different from that shown in figures 3 and 4.

From experience the conclusion would be reached that at 1 p.p.m. phosphate there is slight danger of a phosphorus deficiency in the leaves, but above this concentration there is an adequate phosphorus supply for vegetative (non-fruiting) growth. Excellent top growth resulted at all concentrations above 5 p.p.m. but the roots suffered at the higher concentrations.

It will be noted by comparing the growths of the lemon cuttings in figures 1 to 4 that the longest roots were produced in the low range of phosphate concentration and the shortest roots in the highest range. This could hardly be due to the osmotic effect of the culture solution because the original solution contained approximately 1455 p.p.m. total solids, and the extreme variations did not exceed roughly 200 p.p.m. Darkening of the roots occurred in both of these ranges of phosphate concentration, but root breakdown occurred only in the highest range.

Above the range in which deficiency symptoms are seen, there is always the possibility of confusing excellent top growth at the moment with permanently healthy growth during which fruit production takes place. It could be said that excellent cuttings were grown at the 5.0, 7.5, and 10.5 p.p.m. phosphate concentrations; with other conditions of the solution favorable, these concentrations do not appear excessive.

The photographs illustrate the fact that there is no sharp division line as to what is the best phosphate concentration for growth, but rather that there is a range of concentrations that give equally good results. The best concentration range of phosphate for the growth of citrus holds only for that particular solution, degree of aeration,' state of growth, and environmental condition. Variation in any one of these and other factors may cause a shift in the range of favorable concentration. In fact, growth frequently may be greatly benefited by a deficiency of phosphate, but the degree of such benefit has its injurious limits. Young citrus cuttings grow best in a culture solution lacking phosphate, but later in their growth they are greatly helped by the presence of considerable phosphate. Figures 1 to 4 show the gross symptoms of phosphorus deficiency; at close range they are still detectable at the 1 p.p.m. phosphate concentration.

Other investigators (11) have obtained a maximum growth of barley in culture solutions containing 1 p.p.m. phosphate; corn and beans at 0.5, and also at 0.25 p.p.m.; and sorghum and tomatoes at 0.5 p.p.m. It is conceivable that larger culture vessels and more frequent changes of solutions might have permitted better growth at the phosphate concentrations used with Lisbon lemon cuttings. However, no encouraging signs were seen in this direction when a reduced number of cuttings were used per culture and when more frequent changes of solution were made. PARKER and PIERRE (8) and also TIDMORE (11) have not as yet obtained good growth in cultures at concentrations comparable with those in the soil solutions of rather productive soils.

In the absence of available phosphates in the soil, but with an abundance of iron and aluminum, HOFFER and CARR (6) found corn roots susceptible to root rots. The nature of the plant appears to be important in phosphate nutrition. PFEIFFER, SIMMERMACHER, and SPANGENBERG (9) found that large applications of readily soluble phosphates were beneficial to oats but were injurious to buckwheat and lupine.

The role of phosphate in biological oxidations has been discussed by LYON (7) and by BARMORE and LUCK (1). It is possible that at high phosphate concentrations there is an increase in the respiration

of citrus roots, and under such conditions greater aeration may be required.

Reference to unhealthy roots accompanying high phosphate in unaerated culture solutions has already been made. Brief considera-tion of additional cultures makes it clear that aeration is of the greatest importance for citrus roots, especially when the phosphate concentration is high. The aeration of the roots by frequent changes of solution with the daily addition of aerated distilled water to make up the water losses from shallow culture pans is inadequate under these high phosphate conditions. Figure 5 shows lemon cuttings grown in a culture solution containing at all times approximately 105 p.p.m. phosphate (the iron being added just prior to changing the solution), while figure 6 shows lemon cuttings grown in a solution lacking phosphate to which was added 5–10 gm. of insoluble ferric phosphate. Both culture solutions were vigorously aerated and were contained in Swedish enamelware pans 18 inches in diameter at the top, 13 inches in diameter at the bottom, and 6 inches high.

In both cultures there was excellent top and healthy root growth. The use of abundant aeration prevented the roots of the cuttings grown with 105 p.p.m. phosphate or with insoluble ferric phosphate from becoming unhealthy up to this stage of growth. Because the cuttings require both iron and phosphate, the solubility of the in-soluble ferric phosphate is thereby assumed to be accelerated. Dur-ing hot weather under glasshouse conditions, the mature leaves of the ferric phosphate culture burned somewhat, which points to a possible inadequate phosphorus nutrition from this source under these particular environmental conditions. The top growth appears to be more extensive and the root system more finely divided when the phosphate supply is just barely adequate, as shown in figure 6. However, the actual fresh and dry weights of the leaves and twigs per cutting were somewhat less when the ferric phosphate was used.

These aerated cultures were grown for many months and their excellent growth makes it appear that phosphate nutrition in citrus may be intimately related to the processes of oxidation. These and other experiments suggest that the roots of citrus cuttings require either more oxygen or withstand less carbon dioxide than is ordi-narily assumed, and that there may be a relation between the re-

Figs 5, 6 —Lemon cuttings grown in aerated culture solution fig 5, approximately 105 p p m phosphate present at all times, fig 6, about 10 gm of insoluble ferric phosphate used as sole source of phosphate

sponse to a given fertilizer element and the degree of root aeration. These are important problems for future investigation.

A grouping of the cuttings (figs. 1–4) at the time of harvest, according to the phosphate concentration, simplifies the consideration of the percentages of phosphorus and other constituents of the dry matter in the various portions of the cuttings. In table II the data show that the percentages of phosphorus increase with increasing phosphate concentrations in the culture solution.

TABLE II

PHOSPHORUS CONTENT IN RELATION TO THAT OF SUGAR IN
VARIOUS PORTIONS OF CUTTINGS*

PO₄ IN CULTURE SOLUTION (P.P.M.)	PHOSPHORUS AS PERCENTAGE OF DRY MATTER				REDUCING SUGARS AS PERCENTAGE OF DRY MATTER			NON-REDUCING SUGARS (CHIEFLY SUCROSE) AS PERCENTAGE OF DRY MATTER		
	ORIGINAL LEAVES	NEW LEAVES	NEW SHOOTS	NEW ROOTS	NEW LEAVES	NEW SHOOTS	NEW ROOTS	NEW LEAVES	NEW SHOOTS	NEW ROOTS
0...........	0 0775	......	0.0563	0.1000	......	0.27	0 84	......	0.53	0.73
0.0125 to 0.075....	.0850	0.0938	.0585	.1035	1.94	1 05	.74	1.46	2.34	1.50
0.1 to 0.5..	.0863	.1063	.0663	.1250	1.83	0.90	.92	1 47	1 52	1.22
1 to 10.5...	.0975	.1750	.1219	.1900	3.60	1.66	.78	2.12	2.71	2.38
15.75 to 31.5.....	.1225	.2188	.2041	.2750	2.97	1.50	.71	3.68	3.68	4 55
105........	0.1300	0.2469	0.2375	0.4750	2.66	1.99	0.80	3.99	2.15	3.08

* Cuttings grown from July 3 to December 19, 1934, in solution cultures at different phosphate levels.

The culture solution employed contained 718 p.p.m. nitrate, which is large when compared with the phosphate concentrations used. It is reasonable to assume that the use of large volumes of a culture solution containing much less nitrate would permit better growth at the low concentrations of phosphate in which the cuttings showed symptoms of phosphorus deficiency. Knowledge of the actual phosphorus content of the soil solution is therefore particularly of value, and perhaps of value only, in relation to the concentration of other elements such as nitrate.

Table II also shows the percentages of reducing and non-reducing sugars in the dry matter of the cuttings. In this table the new mature

leaves of the cultures grown without phosphate were not analyzed but were frozen and the juice was extracted under 10 tons of pressure. The juice showed a pH value of 6.06 at 23° C. by the use of the quinhydrone electrode. Leaves of the cultures grown in solutions containing 105 p.p.m. phosphate treated in a similar manner at the same time showed a value of pH 6.52. Phosphate deficient leaves therefore have a greater acidity than have healthy leaves. In this respect they resemble mottled leaves which contain an excess of phosphorus.

The increase of acidity brought about by a deficiency of phosphorus confirms the results of ECKERSON (4) on tomato leaves. These results are of interest because TASAKI, INOUE, and MATSUOKA (10) found in connection with fertilizer experiments with satsuma oranges that the omission of phosphate brought about not only a decreased yield but also an increased acidity in the fruit. It would also be of interest to know whether an excess of phosphate would bring about increased acidity in fruit.

In table II, if we assume the non-reducing sugars as being chiefly sucrose, a relation appears to exist not only between the percentages of phosphorus in the culture solution and in the dry matter of the leaves, but also between these percentages and those of sucrose.

Analyses were made of the ash of the lemon cuttings and the data are presented in table III. The percentage of total ash in the new mature leaves was least at the 1 to 10.5 p.p.m. phosphate concentration and greatest at the lowest concentrations of phosphate (0.0125 to 0.075 p.p.m.). COLBY (2) previously found that the leaves of French prune tree cultures lacking phosphate were high in ash.

The lowest percentages of calcium and potassium in the mature new leaves were found also at the 1 to 10.5 p.p.m. phosphate concentration, while the highest percentages were found at the lower phosphate concentrations in which deficiency symptoms were evident. These results agree with those of ECKERSON (4) on tomato plants and of DAVIS (3) on fruit trees.

The total nitrogen and nitrogen (in the form of nitrate) in the dry matter of the lemon cuttings were determined by the Kjeldahl and reduction methods, respectively, and the results are shown in table IV. In the leaves and shoots the percentages of total nitrogen are

TABLE III

TOTAL ASH, CALCIUM, MAGNESIUM, SODIUM, AND POTASSIUM CONTENT OF LISBON LEMON CUTTINGS*

PO₄ IN CULTURE SOLUTION (P.P.M.)	TOTAL ASH AS PERCENTAGE OF DRY MATTER			CALCIUM AS PERCENTAGE OF DRY MATTER			MAGNESIUM AS PERCENTAGE OF DRY MATTER			SODIUM AS PERCENTAGE OF DRY MATTER			POTASSIUM AS PERCENTAGE OF DRY MATTER		
	NEW LEAVES	NEW SHOOTS	NEW ROOTS	NEW LEAVES	NEW SHOOTS	NEW ROOTS	NEW LEAVES	NEW SHOOTS	NEW ROOTS	NEW LEAVES	NEW SHOOTS	NEW ROOTS	NEW LEAVES	NEW SHOOTS	NEW ROOTS
0.0125 to 0.075	13.34	6.77	8.56	2.25	1.38	1.53	0.44	0.17	0.21	0.22	0.54	0.17	4.08	2.01	1.66
0.1 to 0.5	12.14	6.51	9.12	2.33	1.33	1.36	0.41	0.10	0.23	0.21	0.48	0.19	3.27	1.88	2.16
1 to 10.5	10.34	6.05	10.52	2.06	1.21	1.28	0.37	0.12	0.30	0.14	0.19	0.22	2.72	1.65	2.64
15.75 to 31.5	10.56	7.01	8.77	2.09	1.05	0.94	0.34	0.13	0.55	0.15	0.16	0.23	2.77	1.42	2.29
105	11.07	6.32	10.74	2.11	1.13	1.13	0.36	0.14	0.51	0.22	0.26	0.70	3.05	1.78	0.78

* Cuttings grown from July 3 to December 10, 1934, in solution cultures at different phosphate levels.

greatest at the lower concentrations of phosphate, which is in agreement with the results of COLBY on French prune trees.

The percentages of nitrate nitrogen in the leaves and shoots are highest in the cuttings grown with the least phosphate. ECKERSON and others secured similar data for tomato plants. The results of these investigators and those reported in table IV show that when phosphorus is deficient the reducase activity as described by ECKERSON evidently suffers a great reduction because the absorbed nitrate

TABLE IV

TOTAL AND NITRATE NITROGEN CONTENT OF LISBON LEMON CUTTINGS*

PO₄ IN CULTURE SOLUTION (P.P.M.)	TOTAL NITROGEN AS PERCENTAGE OF DRY MATTER			NITRATE NITROGEN AS PERCENTAGE OF DRY MATTER		
	NEW LEAVES	NEW SHOOTS	NEW ROOTS	NEW LEAVES	NEW SHOOTS	NEW ROOTS
0.0125 to 0.075..........	3.54	1.26	2.59	2.29	0.92	2.31
0.1 to 0.5.........	3.62	1.12	3.11	1.65	.42	2.56
1 to 10.5......	2.77	0.99	3.56	0 49	43	2.27
15.75 to 31.5............	2.60	0.98	4 01	0 36	27	....
105....................	2.75	0.32	3.10	0 44	0.53	....

* Cuttings grown from July 3 to December 19, 1934, in solution cultures at different phosphate levels.

remains largely unchanged in the tissues. For this reason, in agreement with ECKERSON, trees grown with abundant nitrate but with deficient phosphorus are essentially nitrogen starved plants.

Summary

1. A study was made of the concentration of phosphate in the culture solution in relation to the growth and composition of Lisbon lemon cuttings. It was found essential to keep iron out of the solution during intervals in which the phosphate concentration was being maintained.

2. The cuttings were grouped into certain ranges of phosphate concentration in order to simplify the harvesting and analytical analysis.

3. Cuttings grown in solution cultures containing from 0 to 0.2 p.p.m. showed symptoms of phosphorus deficiency regardless of the frequency of change of solution. Slight symptoms of phosphate de-

ficiency occurred at 1 p.p.m. phosphate but none at 2 p.p.m. The longest roots were produced in the low range of phosphate concentration and the shortest roots in the highest range.

4. Lemon cuttings grow well in solutions containing 105 p.p.m. phosphate when such solutions are vigorously aerated. Aeration appears to be an important factor in the phosphate nutrition of citrus.

5. The percentages of phosphorus in citrus leaves in the field decrease as the maturity of the leaves increases. However, in the mature original leaves of the cuttings the percentages were increased with increasing phosphate concentrations in the culture solution.

6. The percentages of reducing sugars in the mature leaves (not the original leaves of the cuttings) are at a maximum at the phosphate concentration range of 1 to 10.5 p.p.m., while those of the nonreducing sugars increase with increasing phosphate concentration. A relation appears to exist not only between the percentages of phosphorus in the culture solution and in the dry matter of the leaves, but also between these percentages and those of sucrose.

7. Phosphate deficient lemon leaves resemble mottled leaves (which contain an excess of phosphorus) in having a greater acidity than healthy leaves.

8. The percentages of total ash, calcium, potassium, total and nitrate nitrogen in the new mature leaves were greatest at the lowest concentrations of phosphate (0.0125 to 0.075 p.p.m.). Absorbed nitrate remains largely unchanged in the tissues when phosphorus is deficient. Trees grown with abundant nitrate but with deficient phosphorus are essentially nitrogen starved plants.

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LITERATURE CITED

1. BARMORE, M., and LUCK, J. M., The role of phosphate in biological oxidations. Jour. Gen. Physiol. 15:97–105. 1931.

2. COLBY, H. L., Effects of starvation on distribution of mineral nutrients in French prune trees grown in culture solutions. Plant Physiol. 8:357–393. 1933.

3. DAVIS, M. B., Investigations on the nutrition of fruit trees. Some effects of deficiencies of nitrogen, potassium, calcium, and magnesium, with special

reference to the behavior of certain varieties of apple trees. Jour. Pomol. and Hort. Sci. **8**:316–344. 1930.

4. ECKERSON, SOPHIA H., Influence of phosphorus deficiency on metabolism of the tomato (*Lycopersicum esculentum* Mill.). Contrib. Boyce Thompson Inst. **3**:197–217. 1931.

5. HAAS, A. R. C., Some nutritional aspects in mottle-leaf and other physiological diseases of citrus. Hilgardia **6**:484–559. 1932.

6. HOFFER, G. N., and CARR, R. H., Accumulation of aluminum and iron compounds in corn plants and its probable relation to root rots. Jour. Agr. Res. **23**:801–824. 1923.

7. LYON, C. J., Phosphate ion as a promoter catalyst of respiration. Jour. Gen. Physiol. **10**:599–615. 1927.

8. PARKER, F. W., and PIERRE, W. H., The concentration of phosphate and potash essential for plant growth in culture solutions. Alabama Agr. Exp. Sta. Rpt. 9. 1927.

9. PFEIFFER, T., SIMMERMACHER, W., and SPANGENBERG, M., Solubility of phosphates and their utilization by oats and buckwheat. Landw. Vers. Sta. **89**:203–230. 1916.

10. TASAKI, K., INOUE, Y., and MATSUOKA, N., Fertilizer experiments with satsuma orange. Imp. Hort. Exp. Sta. (Japan) Res. Bull. 13. 1–60. 1930.

11. TIDMORE, J. W., Phosphate studies in solution cultures. Soil Sci. **30**:13–31. 1930.

GERMINATION OF POPULUS GRANDIDENTATA AND P. TREMULOIDES, WITH PARTICULAR REFERENCE TO OXYGEN CONSUMPTION

MILDRED E. FAUST

(WITH ONE FIGURE)

Introduction

Populus tremuloides Michx. and *P. grandidentata* Michx., which are known to reproduce chiefly by means of root shoots, produce seeds in great abundance in parts of the eastern and central United States. In these regions seedlings are not rare, although most of the reproduction, particularly following burns, is by means of root shoots. Where there is sufficient rainfall there are usually thousands of seedlings germinating on the old leaves or moist soil under or near the mature trees. Few if any of these ever reach more than a few inches in height.

P. tremuloides, which is one of the most widely distributed trees in the western hemisphere, as well as *P. tremula* L., a parallel species in the eastern hemisphere both as to habits and distribution, has short lived seeds which are found in abundance only in certain portions of its range. In Sweden, LAGERBERG (8) states that LOWENHJELM found difficulties in germinating the seeds since they remain viable for only three weeks. PEARSON (12), WEIGLE and FROTHINGHAM (13), and others find that seeds of *P. tremuloides* are not viable under normal conditions for more than three weeks. According to BAKER (2) and PEARSON (12), few seeds have been found in Utah. The latter has also found that in central Utah, owing to the short period of viability, it is difficult for seedlings to become established, except in locally wet places, as the surface of the soil is dry and rains are infrequent at the time of seed dispersal. This almost total absence of seedlings of *P. tremuloides* in such regions as Utah, as well as the small number which persist in other sections, raises the question as to the conditions under which germination and growth are possible.

An outstanding example which answers this question in part is

found at Strawberry Lake, Utah (altitude 7560 feet), where aspen is abundant in the adjacent hills. According to data obtained from W. P. Cottam, the reservoir was completed in 1912 and by June, 1913, 6000 acre-feet of water were impounded. In 1922 the water reached the spillway and again a year later. Since 1923 the water has never filled the lake, and today (1933) there are some 4900 acres exposed which were under water in 1923. The sagebrush which covered this shore region was killed through inundation and the barren opened to invasion. In 1928 the writer found intermingled with the dead sagebrush a great number of seedlings of *P. tremuloides*. Here the ground water of the denuded area was the important factor in establishing the aspen seedlings and supplied the deficiency ordinarily encountered in Utah where the climatic factors are the determiners.

From observation and repeated tests it is evident that seeds of both species of aspen will germinate under a varied range of environmental conditions as long as sufficient moisture is provided. Germination continues unhampered when the seeds are totally submerged in water.

Since moisture appears to be a critical factor in the establishment of these seedlings, and since the seedlings are relatively short lived, it was pertinent to study the effects upon viability of drying the seeds for varying lengths of time and thus of prolonging viability. Temperature studies on rate and percentage of germination were made on seeds of various ages and moisture contents, as well as tests of oxygen consumption at a constant temperature.

Seeds of *P. grandidentata*, which are produced in much greater abundance than those of *P. tremuloides* in the immediate vicinity of study, were used in most cases. It was found that there is practically no difference in the characteristics of the seeds of the two species. The catkin of the former is larger, being about 6 inches long and containing approximately 1000 viable seeds. It is produced a few days later than that of *P. tremuloides*, and before the leaves instead of with or after them.

The term "germination" as here used indicates a point at which the hypocotyl has grown out of the seed coat just far enough to raise one end of the seed from the substratum.

Material and methods

COLLECTING.—The seeds used for detailed studies were collected from trees growing in the crevices of the Onondaga limestone in the vicinity of Syracuse, New York. Records at Syracuse since 1922 show that anthesis for both species varies from March 19 to April 12. From 1927 to 1933 *P. tremuloides* seeds matured from May 10 to 20 and those of *P. grandidentata* from May 12 to 27.

The best time for collecting is on a clear calm day; however, seeds collected in the rain have retained 100% viability at 5° C. for at least one year. The seeds used for study were collected from catkins picked from the trees when the seeds were a light straw color. Those collected before reaching the characteristic straw color do not ripen completely and yield approximately 50% germination.

TRANSPORTATION.—The best method found for transporting seeds in the green catkins over long distances was packing them loosely in fine cheesecloth inserted in burlap bags. One lot of seeds was in transit for three days in extremely hot weather and maintained 100% viability after storage at 5° C. for two years.

DRYING.—Within an hour after collection, the catkins were spread out one or two layers thick in large shallow pans out of direct sunlight in an inside room of uniform temperature. In 1932 both an aneroid barometer and a Julien and Friez hygrothermograph were placed in this drying room. The mean atmospheric pressure for the first two weeks of drying (that is, from May 26 to June 8) was 29.92 inches. This varied from 29.60 to 30.25 inches. The mean temperature was 23.61° C. with a variation of from 20° to 26° C. The mean relative humidity of 8:00 A.M. and 12 noon was 48.50% with a range of from 27 to 66%.

The best length of duration of drying is from three to eight days. In 1930, when the largest number of seeds were collected, both species yielded 100% germination when first collected. Tests at 29° C. were made for seeds dried one, two, three, four, five, six, eight, nine, and ten days and then stored at 5° C. Germination percentages for seeds of both species are similar except that the drop in viability comes sooner in *P. tremuloides*. Those of *P. grandidentata* dried one day showed a sudden drop in germination to 47% before fifteen days and were dead in three months. Those dried both nine and ten days

were dead in thirty-six months and those dried both two and four days were dead in forty-five months. The ones dried six days showed 14% germination in forty-five months and those dried both three and five days had 44 % viable after forty-five months.
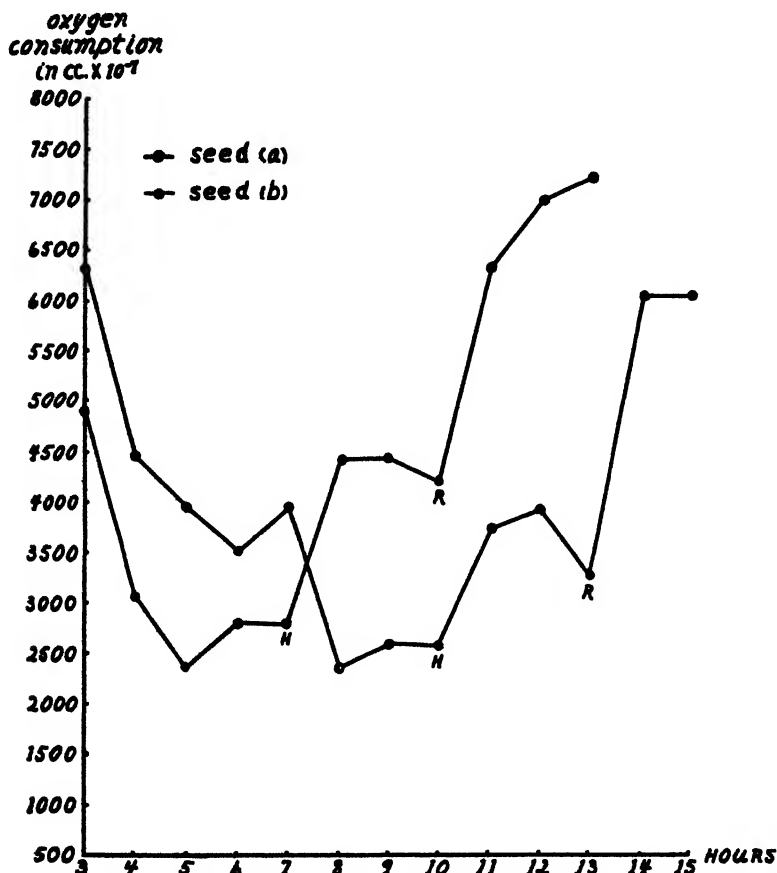
FIG. 1.—Hourly oxygen consumption of single seeds (*a*) and (*b*), *P. grandidentata* 1932, dried six days. Hypocotyl of seed (*b*) emerged two hours before that of seed (*a*), showing individual differences in germination. *H*, hypocotyl; *R*, root hairs.

CLEANING AND GRADING.—When the seeds had dried the desired length of time, the catkins were placed over a wire screen on top of an open box and were separated from the cotton by means of a heavy brush. The seeds which dropped through the screen were blown free

from chaff and graded through screens of 30 and 40 mesh to the inch. Seeds graded with the former screen were used for all the tests except as indicated later.

There is a definite parallel between the curves of germination of small and large seeds. The total percentage of germination of *P. grandidentata* 1928, which was dried four days and planted when thirteen to seventeen days old, showed an average germination of 45% for small seeds as compared with 96% for the large ones.

PLANTING.—All tests of seed germination, unless otherwise noted, were made in petri dishes containing two layers of thoroughly moistened filter paper with the excess water drained off.

The seeds will germinate whenever there is sufficient moisture. At the same temperatures the total percentages of germination are the same regardless of whether or not the seeds are totally submerged in water, or are on filter papers with varying amounts of moisture in similar or different sized dishes.

The percentage of germination was found to be comparable on filter paper (moistened with distilled water), sand of pH 7.0, and humus (in which the aspens grow) of pH 8.5. However, only 82% germinated on sand of pH 4.5 as compared with 98% on the other media.

Twenty-five to one thousand seeds were used for each germination test. In the studies of the effect of temperature upon germination fifty and one hundred seeds were used. It was found that fifty and usually twenty-five gave reliable results when the seeds were of high viability.

STORING.—The seeds were stored in three ways: (1) in open dishes in the laboratory, (2) in corked bottles in the laboratory, and (3) after 1930 in corked bottles at 5° C. in a General Electric refrigerator constant to ±0.5° to 1° C. The seeds occupied from one-half to one-fourth the volume of the bottles, which were opened for ventilation at various intervals.

After eighteen weeks of storage both in open dishes and corked bottles in the laboratory, seeds of both species gave germination percentages of 1. However, *P. grandidentata* 1930 which had been dried both three and five days before storage in a bottle showed 1% after twenty-two weeks. The latter remained 100% viable for twelve

weeks when dried five days, for eleven weeks when dried four days, for nine weeks when dried three days, and for seven weeks when dried nine days. *P. tremuloides* 1930 dried both two and four days before storage in bottles remained 100% viable for thirteen weeks. Any seeds stored in the open retained their 100% viability for only five weeks.

After storage in bottles at 5° C., seeds dried more than two days showed the greatest viability for long periods of time. Those of *P. grandidentata* 1930 which were dried for one day only and placed in a bottle in the laboratory had 76% germination at the end of one week and 0% in two weeks, while the same lot of seeds stored at 5° C. lost viability in twelve weeks.

Until 1930 an icebox of varying temperature was used for low temperature storage. Seeds kept in it fell off more rapidly in percentage of germination than did those kept constantly at 5° C. *P. grandidentata* 1929 and 1930, germinated at 29° C. when thirty-two weeks old, showed 29% for those of 1929 and 98% for those of 1930. After one year *P. grandidentata* 1929, dried five days, gave 1% germination; while that of 1930, also dried five days, gave 91% and was 76% viable after three years.

Cold storage (about 5° C.) of fully matured and dried seed has been found to be important for extensive prolongation of vitality of short lived seeds. NAKAJIMA (10) in his study of *Salix* found that storage under several conditions at a low temperature prolonged vitality. In one instance he obtained 53% germination after three hundred and sixty days. JOSEPH (6) finds that the vitality of parsnip seeds is prolonged decidedly by air drying to a certain low moisture content and subsequent storage in an icebox with frequent ventilation.

Germination results

TEMPERATURE RELATIONS

Germination of both species is not dependent upon light, and with sufficient moisture will take place at any temperature between 0° and 35° C. The General Electric refrigerator was used for germination at temperatures of 5° C. and below and ovens constant to ±0.5° C. for temperatures of 26°, 29°, 32°, 35°, and 38° C.

Seeds dried and stored under similar conditions, even though collected from different trees and in different years, gave similar germination percentages. Seeds of *P. tremuloides* 1930 and 1932 dried three days, before storing at 5° C. for one year, showed germination of 97% at 29° C. Seeds of *P. grandidentata*, stored and germinated under similar conditions, showed 100% in 1930 and 97% in 1932.

. MAXIMUM, MINIMUM, AND OPTIMUM TEMPERATURES.—No particular attempt was made to determine the maximum, minimum, and optimum temperatures for the two species. After exposure for two weeks to the highest temperature used (38° C.), no seeds germinated. When these were transferred to both room temperature and 29° C., however, a number of them were found viable.

Seeds of *P. grandidentata* 1929, dried five days before storage on ice for twenty to twenty-three weeks, gave no germination at 35° C. but gave 39% at 32° C. and 20% at 29° C. A larger number of young seeds will germinate more quickly at 35° C., but complete (100%) germination is attained sooner at 29° and 32° C. Seeds up to one year old which have been frozen for several days and thawed at 5° C., as well as those kept constantly at 5° C., germinate in five days at 5° C.

The largest percentage of germination was attained at both 29°and 32° C. However, the sturdiest appearing seedlings were those from seeds germinated at temperatures from 5° to 29° C. Seedlings of *P. grandidentata* 1929 three weeks old were twice as tall when germinated at 29° as those germinated at 35° C.

Both species of 1930 seeds which were dried for periods of two, three, four, five, and six days and studied for a period of the first five weeks after collection showed curves for the rate and percentage of germination which were practically parallel at the three temperatures, 26°, 29°, and 32° C. The rate was always slower for those germinated at 26° and the average final percentage of germination was 98 as compared with 100 for those germinated at 29° and 32° C.

INITIAL GERMINATION.—Seeds of both species collected from the same trees over a period of six years have been found to show the initial germination in five hours at 29° and five days at 5° C. Occasionally the seeds of *P. tremuloides* germinate in four hours at 29° C. It is found that when seeds are germinated at a higher temperature (29° C.) the initial time becomes greater as the seeds age. For seeds of *P. tremuloides* 1930 dried three, four, and five days and

P. grandidentata 1930 dried four, five, six, nine, and ten days, initial germination at 29° C. occurs in five hours for a period of thirty-two weeks, then it increases to seven hours at forty-nine weeks, to nine hours at one year, and from twelve to twenty-four hours at three years.

P. tremuloides seeds of the same lot as the preceding but dried two days required for initial germination only seven hours at the end of one year; while *P. grandidentata* seeds from the same lot but dried three days required seven hours at forty-nine weeks, eight hours at one year, and from twelve to eighteen hours at three years. The latter retained 100% viability for one year and dropped to 84% in three years.

MICROCHEMISTRY

Microchemical tests were made on two sets of one year old seeds of *P. grandidentata* 1930 with 0% and 92% germination, on one set of *P. tremuloides* 1930 with 90%, on three week old 1931 *P. grandidentata* and *P. tremuloides* with 100%, and on *P. grandidentata* 1931, three day old seedlings.

The contents of the seeds of both species at the same age are comparable. The outer layer of the seed coat of the resting seeds is composed of cellulose and the inner of unknown (yellowish brown) material. The outer wall becomes partially broken down into sugars (mostly sucrose) and starch in the year old viable seeds and decidedly so in the dead ones. In the latter the inner wall breaks down, to a lesser extent, into sugars (some glucose) and starch.

The storage products in the two (or three) cotyledons are fats and proteins, these being in similar amounts and arrangements in the viable young and year old seeds but reduced in amount in the dead seeds.

In the seedlings the fats are scattered throughout the cotyledons but concentrated near the veins. The proteins are near the veins and at the base of the hypocotyl. Small amounts of glucose, fructose, and sucrose are scattered throughout the seedling.

The storage compounds appear to have some bearing upon germination and after-ripening. JONES (5) found that in the genus *Acer* the short lived seeds (*A. saccharinum*) have storage compounds composed mainly of sugars, while the comparatively long lived ones which require after-ripening (*A. saccharum*) have compounds of pro-

teins and fats. Here aspen seeds, with storage compounds of fats and proteins, are found to be short lived, at least in temperate climates. Upon submission to low temperatures, however, they become comparatively long lived.

MOISTURE CONTENT

Moisture determinations of seeds of *P. grandidentata* 1932 were made from averages of two lots of seeds of 1 gm. each which were ground fine in an agate mortar. They were then dried in a vacuum desiccator over concentrated sulphuric acid and kept in a constant temperature oven at 60° C. The moisture percentages after the seeds had been in storage twelve weeks in closed bottles at 5° C. are shown in table II.

When the seeds are heated above 60° C. there is decided discoloration, but from a few tests, sufficient only to give a suggestion, it appears that a higher temperature does not appreciably alter the percentage of moisture of those air dried between four and eight days. Although the seeds of the two species vary slightly in size, the moisture percentage is similar in both when submitted to the same drying conditions. Seeds of *P. tremuloides* 1930, which were dried at 104° C., seven months after collection showed 8.62% moisture. These had been dried for five days before storage at 5° C.

Both 1931 species were dried in a vacuum oven at 79° C. Those of *P. tremuloides*, which were dried five days before storage and the moisture determinations made after they were twenty-six days old, showed 8.83%. Those dried four days before storage and tested after nineteen days, showed 8.85% moisture. *P. grandidentata*, dried for six days before storage and tested when fifteen days old, showed 8.45% moisture. The 1931 seeds of *P. tremuloides* were 100% viable and those of *P. grandidentata* were only 50% viable.

Other writers have also found that longevity of seeds is increased by reduction in the moisture content. JOSEPH (6) found that for parsnip seeds, somewhere below 6.13% moisture at room temperatures appeared to be the "critical moisture content."

Oxygen consumption

A Thunberg microrespirometer, modified by FENN (1927) and later by OBRESHKOVE (OBRESHKOVE and BANTA 11), was used for the study of the oxygen consumption of both seeds and seedlings.

The extreme sensitiveness of the apparatus is apparent by its capacity for measuring accurately and directly the oxygen consumption of ten resting seeds or a single germinating seed of *P. grandidentata*, the average weight of each being 0.0000941 gm.

Attempts were made to use KRAJNIK'S (7) microrespirometer, an apparatus first used on plants (strawberry fruits) in this country by GERHART (3). Owing to its large size, however, and the fact that it could not be entirely submerged in the water bath, it lacked the sensitivity necessary for reactions of very small objects.

The microrespirometer, which is composed of two glass bottles connected by a capillary containing a kerosene drop, was totally sub-

TABLE I

OXYGEN CONSUMPTION FOR SEEDS OF P. GRANDIDENTATA 1932

MICRORESPIROMETER TEST	OXYGEN CONSUMPTION PER SEED PER MINUTE IN CC. $\times 10^{-7}$				
	SEED 0	SEED 2	SEED 4	SEED 6	SEED 8
First hour.............	53 499	25.309	10 823	14.640	9 656
Second hour....... . . .	52.642	19.701	14 485	14 484	14 374
Third hour..	49 371	23 985	17 910	21 804	16 197
Fourth hour...........	48 437	25 153	20.091	20 168	19.076
Average....	50 987	23.537	15 827	17 774	14.826

merged in a Freas waterbath with a thermostat control set at 25° C. and sensitive to ±0.005° C. It was absolutely necessary to keep the temperature as nearly constant as possible. A variation of more than ±0.01° C. caused enough fluctuation in the volume of oxygen consumed to render the results worthless. In order that the microrespirometer attain the exact temperature of the waterbath, it, with its contents of a 2% solution of NaOH and the seed or seeds, was submerged in the bath for an hour before it was closed to the air and then left another hour before readings were taken.   .

For resting seeds readings were taken hourly over a period of four hours (table I). The oxygen consumption is represented in cubic centimeters times 10^{-7} per seed per minute. Seed 0 is the lot of seeds not dried at all, seed 2, that dried two days, etc. The recorded tests are from those lots of seeds which showed 100% germination after removal from the microrespirometer. Care was taken at all times to

handle the seeds as little as possible when removing from storage and placing in the microrespirometer.

The seeds used were those of *P. grandidentata* 1932 which had been dried zero, two, four, six, and eight days. Ten seeds were used and an average of five tests is recorded for each hour. Repeated tests at the immediate close of the drying period and also within two months after they were stored showed comparable amounts of oxygen consumption.

As the moisture content of the seeds decreases the oxygen consumption decreases (tables I and II). Such a correlation has been found for certain other seeds by a number of workers, notably BAILEY (1), JAQUOT and MAYER (4), and MAIGE and NICOLAS (9).

TABLE II

OXYGEN CONSUMPTION AND MOISTURE ABSORPTION FOR SEEDS OF P. GRANDIDENTATA 1932 DRIED FOR DIFFERENT LENGTHS OF TIME

No. of days dried	Average 4 hours oxygen consumption	Percentage moisture absorbed	Moisture content	Percentage germination at tests	Percentage germination at end of one year
0	50.987	. . . .	49 22	100	0
2	23.537	. . . .	30.52	100	0
4	15 827	11.30	9 18	100	67 (poor)
6	17.774	12.07	6 65	100	95 (healthy)
8	14.826	10 02	7.52	100	95 (poor)
73	. . . .	. . . .	6.97	100	. . . .

As noted in table I, there is a gradual increase in the amount of oxygen consumed during the first four hours of the tests of seeds 4, 6, and 8. It was found that these absorbed an appreciable amount of water from the NaOH solution (table II).

The comparatively large amount of oxygen consumed in freshly harvested seeds drops to approximately half after one day of drying. After the third day of drying there is no considerable difference in the amount consumed. Of the 1932 seeds those dried six days are the best from the standpoint of longevity, for after thirty-two months 85% of them were viable as compared with 65% of seed 8 and 17% of seed 4.

The most striking results were obtained when the oxygen consumption of a single seed placed on moist filter paper in the micro-

respirometer was checked at five-minute intervals. The seedlings were all taken from seed 6 which had been in storage at 5° C. for two months.

In order to obtain a relative idea of the oxygen consumed during the first two hours, a seed (*c*) was placed in the microrespirometer for only fifteen minutes before readings were taken. Using round numbers for cubic centimeters of oxygen times 10^{-7}, during the second fifteen minutes it absorbed 13,300; during the third fifteen minutes, 4200; and the fourth fifteen minutes, 1700. Two other seeds (*a*) and (*b*) were left in the microrespirometer for one hour before it was closed and the readings taken for the second hour of germination. Seed (*a*) consumed approximately 47,200 and (*b*) 41,100 cc. times 10^{-7}. Figure 1 illustrates the results of the latter two seeds from the third to the fifteenth hours of germination and the third to the thirteenth hours respectively. Here the results are noted after the seeds and apparatus had been in the bath for two hours, as was done with the resting seeds. These two seeds were chosen because they illustrate individual differences in initial time of germination. The hypocotyl of seed (*b*) was 1 mm. long and the crown of root hairs 0.5 mm. long (one-half final length) at the end of thirteen hours, while it required fifteen hours for those of seed (*a*) to attain the same lengths.

Summary

1. *Populus tremuloides* and *P. grandidentata* produce seeds in great abundance in central and eastern United States. The former seeds mature approximately three days earlier than the latter. From six to ten weeks elapse between anthesis and maturation of the seeds.

2. Seeds collected when a light straw color and dried at once retain their viability the longest.

3. The best length of time for drying is from three to eight days in a room of uniform temperature of about 24° to 25°C. Seeds dried only one day lose viability in thirteen weeks, even when placed at 5° C.

4. Small seeds do not germinate so readily nor retain their viability so long as large ones. This applies to individuals within the species.

5. Seeds showed the highest percentage of germination in media of pH 7.0 to 8.5.

6. Except when the viability is low, fifty seeds are sufficient for showing accurate percentages of germination.

7. A constant low temperature (approximately 5° C.) was found best for prolonging viability.

8. Seeds have been found to retain 100% viability for as long as thirteen weeks when placed in corked bottles in the laboratory and for five weeks in open dishes. Most of these lose their viability after eighteen weeks but occasionally show 1% at twenty-two weeks.

9. Seeds germinate whenever there is sufficient moisture (even when submerged) between temperatures of 0° and 35° C. Freezing for several weeks does not appear to injure the seeds when they are thawed at 5° C.

10. The largest percentage of germination is obtained at 29° and 32° C.; 35° to 38° C. appears to be too high but may only retard germination. The sturdiest seedlings are from seeds germinated at temperatures from 5° to 29° C.

11. The time for initial germination is usually five hours for seeds germinated at 29° and 32° C. This increases as the seeds age. Short initial germination time is correlated with high viability.

12. The seeds of both species are fatty and contain some proteins.

13. Seeds lose moisture rapidly during the first two days of drying. After three days there is little loss.

14. Oxygen consumption was studied by means of a microrespirometer of the closed system so sensitive that a temperature constant to ±0.005° C. was necessary for accurate results.

15. The extreme sensitiveness of the apparatus makes it possible not only to measure the oxygen consumption of ten resting seeds at half-hour intervals, but also that of a single germinating seed at five-minute intervals.

16. Oxygen consumption is high when the seeds are viable and contain much water. This decreases with the decrease in water content of the seeds.

17. The very great amount of oxygen consumed when the germinating seed first absorbs water drops to a minimum two hours before the hypocotyl emerges from the seed coat. It then rises rather abruptly for an hour, drops slightly in the next two hours, after which the crown of root hairs emerges. There is again a rather abrupt rise for an hour and then it becomes comparatively uniform.

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LITERATURE CITED

1. BAILEY, C. H., Respiration of shelled corn. Univ. Minnesota Agr. Exp. Sta. Tech. Bull. 3.1-44. 1921.

2. BAKER, F. S., Aspen in the central Rocky Mountain region. U.S.D.A. Bull. 1291 1-46. 1925.

3. GERHART, A. R., Respiration in strawberry fruits. BOT. GAZ. 89:40-66. 1930.

4. JAQUOT, R., and MAYER, A., Hydration et respiration des graines. Ann. Physiol. Physicochim. Biol. 2:408-425. 1926.

5. JONES, H. A., Physiological study of maple seeds. BOT. GAZ. 69:127-152. 1920.

6. JOSEPH, HILDA C., Germination and keeping quality of parsnip seeds under various conditions. BOT. GAZ. 87:195-210. 1929.

7. KRAJNIK, B., Über eine Modifikation des Microrespirationsapparates. Biochem. Zeitschr. 130:286-293. 1922.

8. LAGERBERG, Om uppdragning av aspforöplantor. Skogsvårdsföreningens. Tidskrift. 125-142. 1922. Rev. Jour. For. 21:627-630. 1923.

9. MAIGE, A., and NICOLAS, G., Researches sur l'influence des variations de la turgescence sur la respiration de la cellule. Rev. Gén. Bot. 22:409-422. 1910.

10. NAKAJIMA, Y., Weitere Untersuchungen über die Lebensdauer der Weidensamen. Sci. Rept. Tôhoku Imp. Univ. Biol. 1:261-275. 1926.

11. OBRESHKOVE, V., and BANTA, A. M., A study of the rate of oxygen consumption of different *Cladocera* clones derived originally from a single mother. Physiol. Zool. 3:1-8. 1930.

12. PEARSON, G. A., The rôle of aspen in the reforestation of mountain burns in Arizona and New Mexico. Plant World 17:249-260. 1914.

13. WEIGLE, W. G., and FROTHINGHAM, E. H., The aspens: Their growth and management. U.S.D.A. For. Serv. Bull. 93.1-34. 1911

CHROMOSOME BEHAVIOR IN AGROSTIS NEBULOSA

FRED W. TINNEY

(WITH TWENTY-FOUR FIGURES)

Introduction

·One of the most important regularities of chromosome behavior is the side-by-side alignment of their homologous parts during the heterotypic prophases. Thus in a typical diploid, individual meiotic chromosomes associate in pairs; in a polyploid or polysomic, the existence of more than two homologous chromosomes commonly leads to multivalent associations. Extensive cytological and genetical studies of structural changes in chromosomes, particularly of reciprocal translocations of parts of non-homologous chromosomes in several genera, confirm the general rule that homologous parts tend to pair in meiosis.

The behavior of chromosomes having reciprocal translocated segments, as shown by cytological observation, is in general the formation in late diakinesis of an open ring or chain of four or more chromosomes joined by terminalized chiasmata. The random distribution of the catenated chromosomes in metaphase I results in a chromosomal deficiency in a certain proportion of the spores, and in the great majority of reported cases such a deficiency is lethal to the gametophyte.

Little is known from previous accounts of the cytology of *Agrostis*. In 1928 AVDULOW (see 12) found forty-two chromosomes in root tips in *A. alba* and twenty-eight chromosomes in those of *A. vulgaris*.

Investigation

For this study, seed of *Agrostis nebulosa* Boiss. & Reut. was obtained from Vaughan's Seed Company, Chicago, Illinois, and grown in the botanical greenhouses at the University of Wisconsin. Sax's modification of Navashin's fluid gave the best fixation for cytological observation. Sections of flowers and root tips were cut 10 μ in thickness and stained with Heidenhain's iron-alum haematoxylin.

The fourteen somatic chromosomes are shown in figures 1 and 2. Differences in length and form are not as yet sufficiently determined
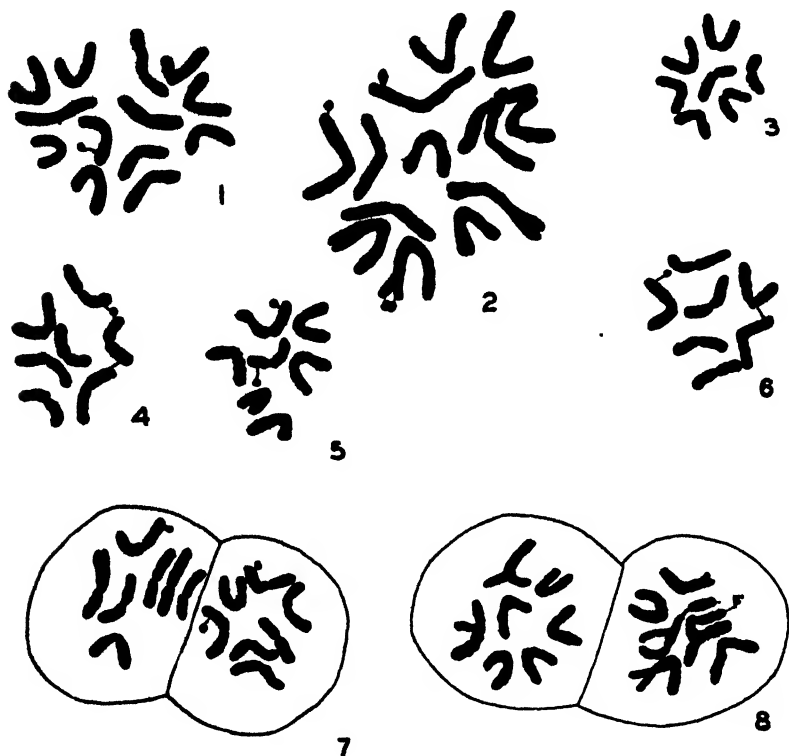
Figs. 1–8.*—Figs. 1, 2, equatorial plates of root tips; figs. 3–8, same of homoeotypic divisions: Fig. 1, thirteen chromosomes; one chromosome with a subterminally attached fragment (one chromosome not shown). Fig. 2, fourteen chromosomes; three chromosomes each with an attached fragment. Fig. 3, seven chromosomes; no fragments. Figs. 4–6, seven chromosomes; two chromosomes each with an attached fragment (one fragment proper is hidden in figs. 4, 6). Fig. 7, sister cells; one cell has one, the other two chromosomes each with attached fragment. Fig. 8, sister cells; seven chromosomes in one cell, in the other six and two fragments (f), one being attached to end of another chromosome. ×3662.

* All figures drawn with the aid of an Abbé camera lucida at table level. A Zeiss 2 mm. apochromatic objective, N.A. 1.3, and a Spencer 30× compensation ocular were used.

to identify each chromosome pair. However, small differences in length and in the position of spindle fiber attachments may, after detailed study of many figures, make such identification possible.

The morphology of the chromosomes is most easily observed in the homoeotypic metaphases (figs. 3–7), although in these figures the chromosomes are smaller than in mitoses in root tips. In general, three chromosomes of the haploid complement are slightly shorter than the other four; of the three shortest chromosomes two have arms of slightly unequal length. The other five (one short, four long) apparently have arms of equal length. No satellites of the ordinary type have been observed. The laterally attached structures, of which there are three in the root tips of some plants (figs. 1–2), and in homoeotypic divisions (figs. 4–7), are probably derived from chromosome fragments. They show no relationship to the nucleolus.

In this paper only the chromosome behavior from diakinesis onward will be considered, except as certain evidence presented by diplotene stages may aid in interpreting configurations in diakinesis.

Most microspore mother nuclei in late diakinesis present a typical condition of seven bivalents, each composed of two chromosomes associated by terminal chiasmata. In relatively few nuclei, although in a sufficient number to remove any doubt of the validity of the observation, two or three bivalents, usually typical in appearance, are associated together at their ends. Two types of such associations are observed—either a chain of two or three bivalents, or an association of three bivalents joined at a common point. Both types may occur in the same nucleus.

In early diakinesis the two components of each of the seven pairs are associated regularly by interstitial chiasmata (assuming that nodal points in at least some cases represent chiasmata). In some instances a variable number of the regularly formed bivalents are joined end to end in one or two chains. In the nucleus shown in figure 9 there are two chains, one consisting of three and the other of two bivalents. Each of the nuclei shown in figures 13 to 15 (a) contains one chain of two bivalents. Occasionally (fig. 13, a) one free arm of one pair is attached to the nucleolus, the corresponding free arm being joined to the end of one chromosome in another chromosome pair. The relation of the attached chromosomes to the nucleolus is not clear. In some nuclei one end of a chromosome pair is either connected by a fiber with the nucleolus (fig. 17) or is lying over the latter. In one nucleus, of which two chromosome pairs are shown in
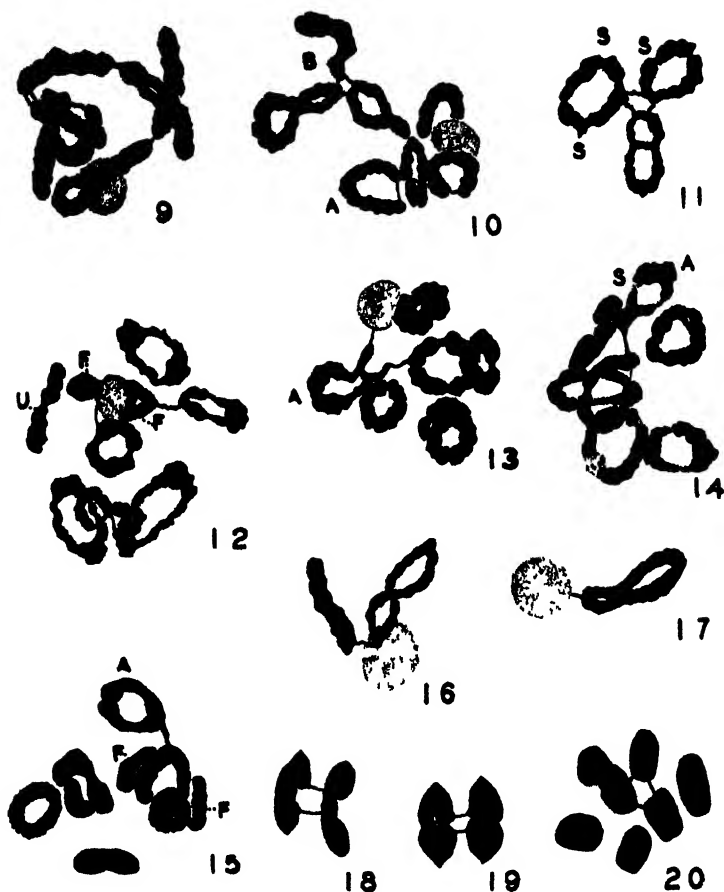
FIGS. 9-20. —Figs. 9-17, diakinesis; figs 18-20, equatorial plates of heterotypic divisions: Fig. 9, early diakinesis; two chains of bivalents, one consisting of three, the other of two bivalents; two bivalents are separate. Fig. 10, one tri-association (*b*) and one chain of two bivalents (*a*); two bivalents are separate. Fig. 11, three bivalents in tri-association; interstitial chiasma in one pair; spindle fiber attachment (*s*). Fig. 12, six bivalents, one univalent (*u*), two fragments (*f*). Figs. 13, 14, one chain of two bivalents (*a*), five separate bivalents; interstitial chiasma in fig. 13 (*a*). Fig. 15, one chain of two bivalents (*a*); two unattached fragments (*f*). Fig. 16, one chain of two bivalents; one pair associated with nucleolus. Fig. 17, pair associated with nucleolus. Figs. 18, 19, side view of two pairs showing respective ends connected. Fig 20, polar view of tri-association. ×4500.

figure 16, the ends of one pair are lying over the nucleolus and also are connected to the end of a chromosome of the other pair. It is possible that the chromosome pairs here concerned are the same as those shown somewhat similarly arranged in figure 13 (*a*).

The second type of configuration (figs. 10, *b*, 11) consists of three bivalents so arranged that the corresponding ends of both chromosomes of one pair are associated respectively with the ends of chromosomes of two other pairs. For convenience this type of configuration will be referred to as a tri-association. A tri-association (*b*) and
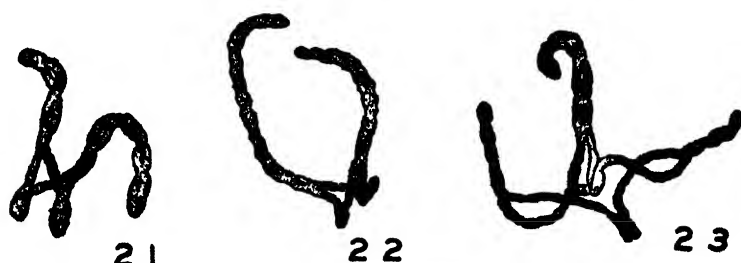
FIGS. 21–23.—Diplotene strands which may lead to bivalent association shown in diakinesis: Figs. 21, 22, two bivalent strands; free ends of one conjugated with free ends of other. Fig. 23, three bivalent strands; free end of one chromosome of pair conjugated with one free end of one chromosome of second pair, the free end of the other chromosome conjugated with free end of one chromosome of third pair. ×4500.

a chain of two bivalents (*a*) may occur in the same nucleus (fig. 10). It is possible that in some nuclei two of these pairs (instead of three) are united, thus forming a chain of two rather than a tri-association.

The study of diplotene figures reveals a condition of pairing which by subsequent terminalization of chiasmata could result in the diakinetic conditions just described. Each thread is clearly double, the pairing in most cases being complete along the entire length. In a few nuclei observed in diplotene, two bivalent threads were regularly paired on both sides of the median spindle fiber attachment region except for the extreme portion at one end. The "free" ends of one diplotene thread were conjugated with the "free" ends of the other (figs. 21, 22). Such a condition might be expected to lead to a chain of two bivalents in diakinesis.

In one nucleus (fig. 23) three bivalent strands with free ends were seen so arranged as to produce in diakinesis a tri-association. Of the

two free ends of a given pair, one was conjugated with a free end of the second pair, the other with a free end of the third pair.

It is evident that the interstitial chiasmata terminalize on both sides of the attachment region in each pair. In some instances, however (figs. 11, 13, a), one chiasma in one of the catenated bivalents had apparently failed to terminalize, whereas in the other bivalent or bivalents terminalization was complete. In many bivalents, both in catenated and in separate pairs, a completely terminalized chiasma is pulled out into a chromatic thread. Since there are interstitial chiasmata regularly on both sides of the fiber attachment region, terminalization could not result in an open ring of four chromosomes without the rupture of a chromatid, which our present knowledge of chromatid behavior renders improbable. The conjugation of the free ends of the two or three bivalent threads probably does not result in a chiasma; it persists, however, in consequence of the formation of a common matrix about the conjugated ends. The force leading to terminalization within each pair pulls the free ends apart; the resultant connection is a fiber having its origin from the matrix.

BEADLE (1) has shown that translocated portions of homologous parts of *Euchlaena* and *Zea* chromosomes conjugate in zygotene and are associated after diplotene by no apparent chiasmata. He suggests that post-diplotene association may be due to the common matrix of the two chromosomes, and that, following repulsion, the daughter chromosomes are held together at their ends by a fiber originating from the matrix.

Another peculiarity of chromosome behavior in *Agrostis nebulosa* is the occasional occurrence of univalents and fragments. In one nucleus (fig. 12) there were six typical bivalents, one univalent (*u*), and two fragments (*f*). Each fragment was joined to the end of a chromosome of a different pair. The two fragments taken together probably represent the homologue of the univalent. Another nucleus (fig. 15) contains six pairs and two unattached univalents (*f*). Occasionally in metaphases of homoeotypic divisions fragments of a similar nature occur. In the sister cells (fig. 8) one cell has seven chromosomes; the other six and two fragments (*f*), one of which is attached to the end of another chromosome.

In equatorial plates and in metaphases, the chromosomes lie too

closely together for multivalent associations to be readily observable; in many instances too the connections are probably broken by this time. However, figures 18 and 19 show two instances in each of which two neighboring pairs are attached by fibers terminal to their individual chromosomes. A polar view of a tri-association in the equatorial plate stage is shown in figure 20.

The seven pairs of chromosomes are regularly arranged at the equator of the spindle. The connections between the pairs observed in diakinesis, if they now exist, do not affect chromosome disjunction.

In the metaphases of homoeotypic divisions and in the mitoses in root tips an occasional chromosome bears a subterminally attached fragment. In one plant studied in detail three such chromosomes were the largest number observed in any root tip nucleus; it is possible, however, that fragments borne on other chromosomes were hidden. In the same plant, homoeotypic metaphases occasionally show a total of three chromosomes, each with an attached fragment, on two sister spindles. In some sister cells (figs. 4–6) there are two "fragment" chromosomes (one fragment proper is probably hidden in cells shown in figures 4 and 6); in others there is one; and in others, none (fig. 3). In the two sister cells shown in figure 7, one cell has one fragment chromosome, the other has two. If three such chromosomes are present in the spore mother cell, a 0–3 or 1–2 distribution would be expected to occur in the heterotypic division. In some plants no fragments have been observed; and in one plant only one chromosome could be found with an attached fragment.

Laterally attached fragments have been reported previously in *Tradescantia* (5), *Allium* (9), and *Festuca elatior pratensis* (10).

Several hundred microspores and pollen grains in different stages of development were studied and in no case was an irregular nuclear or cell division observed. A count of the mature pollen grains showed about 3 per cent abortion.

Discussion

Association in meiosis of four or more "non-homologous" chromosomes has been described in a number of genera, including several forms of the Gramineae (*Zea mays*, 2, *Briza media* and *Anthoxanthum*

odoratum, 7, 8, *Festuca pratensis*, 10). In nearly every case thus far reported the chromosomes are joined terminally into a ring or chain. In those cases of this nature which have been studied sufficiently to present cogent evidence on the basis of the hypothesis of segmental interchange, at least half of the chromosomes in the ring (one of each pair) have each a relatively large interchanged segment. Alternate disjunction of such catenated chromosomes results in the production of complete chromosome complements, and hence in the viability of the spores containing them; adjacent disjunction produces some chromosome combinations that are non-viable because of a deficiency of a relatively large part of a chromosome. It has been shown in *Zea mays*, however, that a small deficiency may be non-lethal or only partially lethal to the gametophyte (3, 4, 11).

HÅKANSSON (6) found in *Pisum* associations of two bivalents in which the chromosomes of one pair were connected by two terminal chiasmata, those of the other pair by one terminal chiasma. He suggests that in such cases small terminal portions of certain chromosomes had been translocated.

As has been seen, conditions of chromosome pairing appear at diplotene in *Agrostis nebulosa* from which both types of bivalent association herein described might be derived. In reference to the chain configurations, the diplotene strands in question are composed each of two chromosomes paired for the greater part of their length in consequence of interstitial chiasmata. The free end of each chromosome is paired with a free end of a chromosome of another bivalent strand (figs. 21, 22). Assuming that only homologous parts of chromosomes pair, and that as in *Zea* the homologous parts conjugate along their entire length, the behavior of these strands leads to the assumption that the terminal portions of two chiefly non-homologous chromosomes had been involved in a reciprocal translocation. There was no evidence of chiasmata in the free arms; apparently they were associated in consequence of their homology until a common matrix formed about them; or if there was a chiasma it had slipped off the end, pulling out the matrix into a fiber. A possible interpretation of this nature based on segmental interchange is shown in figure 24, *A*.

In other diplotene figures three bivalent strands are associated at a common point (tri-association, fig. 23). The end of one chromosome of a pair conjugates with the end of one chromosome of a second pair; the corresponding end of the latter conjugates with the end of
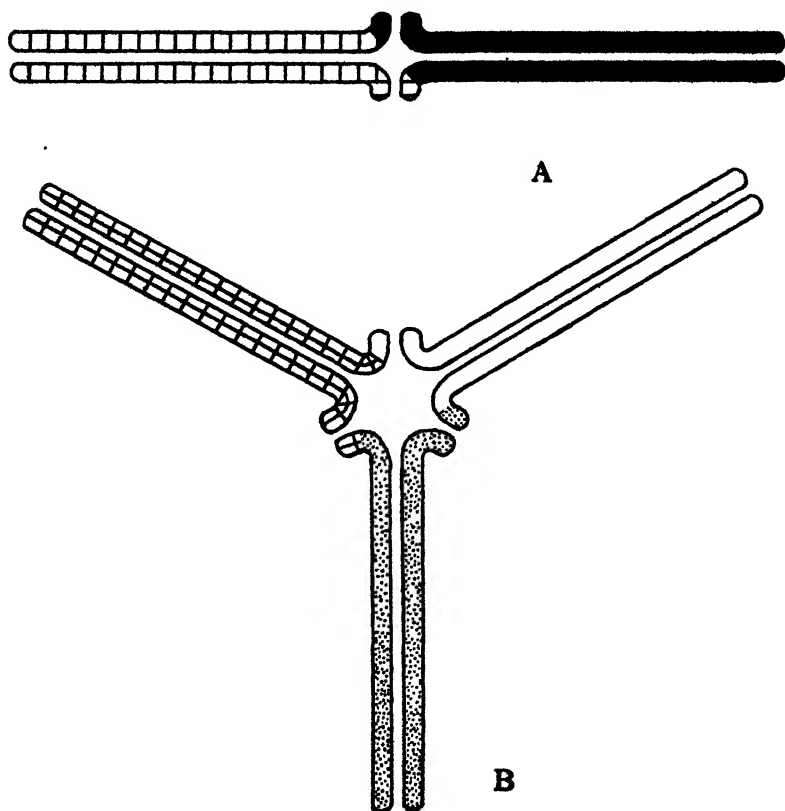
FIG. 24.—*A*, suggested constitution of the two chromosome pairs found in chain at diakinesis; *B*, suggested constitution of the three chromosome pairs found in tri-association at diakinesis.

one chromosome of a third pair. No chiasmata were probably formed in the free ends which apparently remain held together, as in the previous case, by a common matrix. The presumably homologous parts of the chromosomes and their translocations are shown in figure 24, *B*. It is evident that, if the interpretation here indicated

is correct, this is a three-way translocation (tri-translocation). To account for such translocation it is conceivable that the ends of the three non-homologous chromosomes were at some time accidentally in contact, and that in consequence of a twisting torsion the end of each was broken and became attached to one of the other two non-homologous chromosomes.

The subterminally attached fragments observed in the equatorial plates of the homoeotypic and of root tip divisions may in some way be related to the translocation here suggested. The fact that three was the greatest number of fragment chromosomes found suggests the possibility, for which there is no available proof, that these three chromosomes and their respective homologues are the ones found associated in the tri-association and that the fragments are the translocated chromosome parts. It remains possible, however, that the fragments are related to the reciprocal translocation which involved at least two other chromosomes, or that the fragments resulted from an irregular separation of daughter halves of chromosomes and bear no relationship to either of the multivalent associations.

If we assume that reciprocal translocation and tri-translocation have taken place, it is evident that the deficiencies resulting in some microspores are not lethal to the gametophytes. The small amount of aborted pollen observed might well be found in a typical individual.

Summary

1. The haploid chromosome number in *Agrostis nebulosa* is seven.

2. In most instances seven typical bivalents appear in diakinesis, the chromosomes of each pair being connected by two terminalized chiasmata. In a few instances two or three bivalents are joined end to end in a chain; or three bivalents are joined at their ends at a common point (tri-association); both types may occur in the same nucleus. Occasionally univalents and fragments are found.

3. Each diplotene strand is clearly double, the pairing in most cases being complete along the entire length. In a few nuclei observed in diplotene, two bivalent threads were regularly paired on both sides of the median spindle fiber attachment except for a very short portion at one end. The free ends of one diplotene thread were

conjugated with the free ends of the other. Such a condition might be expected to lead to a chain of two bivalents in diakinesis. In one nucleus three bivalent strands with free ends were seen so arranged as to produce in diakinesis a tri-association.

4. Interstitial chiasmata usually terminalize in typical and catenated bivalents on both sides of the spindle fiber attachment. In a few catenated bivalents complete terminalization was not attained. In no case observed was there an open ring of four or more chromosomes in diakinesis in consequence of terminalized chiasmata.

5. It is suggested that a small terminal portion of one chromosome in each of two or three pairs found in a chain has undergone reciprocal translocation, and the extreme end of one chromosome in each of three other bivalents found in a tri-association has undergone a three-way translocation.

6. Laterally attached fragments seen in somatic and homoeotypic divisions are probably constant features of these chromosomes, and may bear some relationship to the translocations here suggested.

7. No appreciable abortion of pollen grains was found.

LITERATURE CITED

1. BEADLE, G. W., The relation of crossing over to chromosome association in Zea-Euchlaena hybrids. Genetics 17:481–501. 1932.

2. BRINK, R. A., and COOPER, D. C., The association of semisterile-1 in maize with two linkage groups. Genetics 16:595–628. 1931.

3. BURNHAM, C. R., An interchange in maize giving low sterility and chain configurations. Proc. Nat. Acad. Sci. 18:434–440. 1932.

4. CREIGHTON, HARRIET B., Three cases of deficiency in chromosome 9 of Zea Mays. Proc. Nat. Acad. Sci. 20:111–115. 1934.

5. DARLINGTON, C. D., Recent advances in cytology. Blakiston. 1932.

phytes in this material agreed closely with Howe's (3) original description of *S. cristatus*. Spores from ten of the *S. cristatus* sporophytes were sown, and some of the resultant sporelings, coming from spores of four sporophytes, were transplanted separately. Among the clones to which they gave rise are both females and males.

It is clear that, as emphasized by Howe, *S. cristatus* is very distinct from the species of the *S. texanus* group. The spore wall bears sinuous ridges which only occasionally anastomose, instead of being regularly areolate; the spores separate early, whereas in the areolate-spored species they remain together in tetrads. Exceptions to the latter rule are certain races of *S. donnellii* whose spores separate in the course of maturation as shown by Siler (9)—not at maturity, as reported by previous investigators. In *S. cristatus*, too, the foot remains attached to the capsule when the latter is removed from the gametophyte; in the other species mentioned, the capsule separates from the imbedded foot. The gametophytes also differ in appearance; the thallus lobes of *S. cristatus* are more rigid and more uniformly entire-margined.

The distinctness of this species suggested a study of its chromosomes in comparison with those of the better known forms. Thallus tips of eight female and seven male clones were fixed and imbedded in paraffin by the usual method. Fixing fluids used were Carnoy's alcohol-acetic, Flemming's medium, the same diluted to four-fifths strength, and Sax's modification of Navashin's mixture. The three latter solutions were used alone, and also after dipping the material in the Carnoy fixative. Sections were stained with Heidenhain's iron-alum haematoxylin, except that some of the material fixed according to Sax's formula was stained by the crystal violet-iodine method as modified by Smith (10). There is little to choose between preparations of material treated in these various ways; all have given usable and mutually corroborative figures.

Observations

FEMALE GAMETOPHYTE

In a polar view of an equatorial plate in a female plant of *S. cristatus* (figs. 6, 7), eight chromosomes are visible. The number is the same as in *S. donnellii*, but none of the eight is distinguished from

the others as is the X-chromosome in the female of the latter species (fig. 12) by its markedly greater length and thickness. Instead, all the members of the female chromosome complement of *S. cristatus* are approximately alike in diameter, although differing in
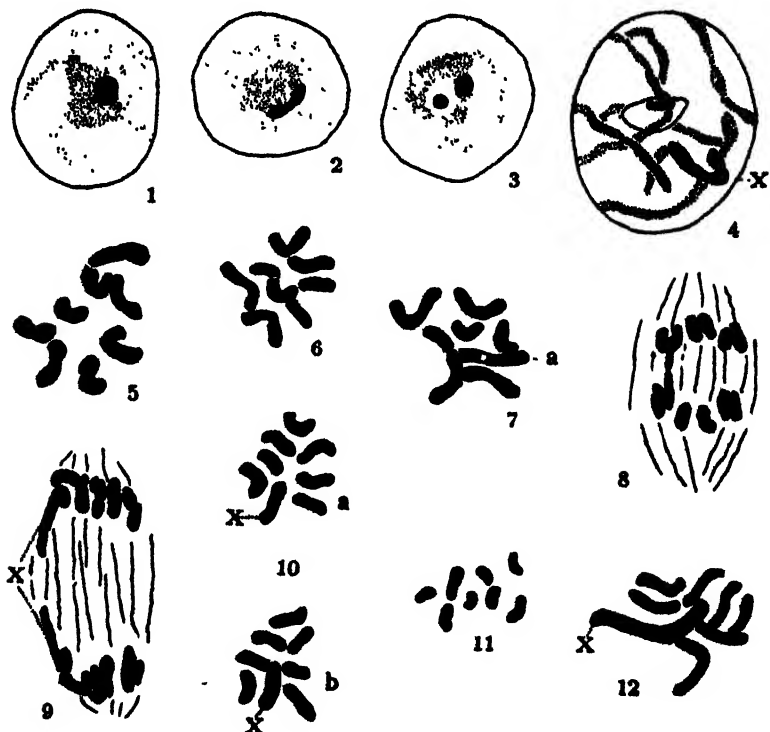
FIGS. 1–12.—Figs. 1–11, *Sphaerocarpos cristatus* ♀: figs. 1–3, resting nuclei, each showing one or two heteropycnotic bodies; fig. 4, prophase nucleus, the X-chromosome heteropycnotic; fig. 5, very late prophase chromosome group; figs. 6, 7, polar views of equatorial plates; figs. 8, 9, anaphases seen laterally; fig. 10, upper (*a*) and lower (*b*) sister anaphase chromosome groups in polar view; fig. 11, anaphase group in polar view. (Figs. 1, 4, 6, 7 from involucral cells; others from thallus cells.) Fig. 12, *S. donnellii* ♀: polar view of equatorial plate (involucral cell). All × c .3600.

length. Their small size and the foreshortening to which some are inevitably subjected render statements as to comparative length necessarily tentative. But from a study of such plates as that shown in figure 7, in which all the chromosomes lie approximately parallel

to the plane of the section, it appears that there may be three relatively long elements, two of medium length, and three short.

Spindle fiber attachments of the short chromosomes, and possibly of one of the long ones, are median; those of the other chromosomes are submedian. The fiber attachment points are commonly marked by constrictions, as various of the figures show; very rarely the constriction region appears achromatic (chromosome a, fig. 7).

At the equatorial plate stage the chromosomes invariably overlap more or less, especially by their ends. In studying even such groups as those shown in figures 6 and 7, which are among the most favorable found, a determination of the chromosome number involves conclusions based upon relative depths of focus. In a very late prophase (fig. 5), however, or still better in polar views of anaphases (figs. 10, 11), the chromosomes are often entirely separate from one another and the count of eight can be made with objective certainty.

Evidently at the equatorial plate stage an X-chromosome cannot be distinguished, although comparison with the chromosome complement of the male makes it certain that one of the chromosomes of the female is an X. There are, however, peculiarities of behavior which at certain stages make possible a reasonably confident recognition of this element.

In regions of the female plant, such as the growing tip, where cells are small and divisions succeed one another at relatively short intervals,[1] if the preparation is sufficiently destained, one small dark-staining body is commonly seen in a resting nucleus. This body, rounded (fig. 1) or elongated (fig. 2), is regularly in contact with the more lightly stained central mass consisting of nucleolus and adherent chromosome substance. Less often two smaller heteropycnotic bodies are present (fig. 3). The condition is precisely similar to that described by TINNEY (11, 12) as occurring in the females of *S. donnellii* and *S. texanus*. In both the latter species the heteropycnotic body has been shown to be a part of the X-chromosome.

[1] Some writers speak of such regions as meristematic. But it must be remembered that all or most of the cells of the thallus of *Sphaerocarpos*, although increasing in size, remain for a long time essentially meristematic, in the sense of being undifferentiated and capable of division. The possibility of the division of relatively old cells is illustrated by the very frequent occurrence of regeneration from various parts of the thallus (6).

Although for obvious reasons this cannot be demonstrated to be the case in *S. cristatus*, there can be little doubt that in this species also the heteropycnotic body represents either a part or the whole of an X-chromosome.

LORBEER (5) apparently considers that there are regularly two heteropycnotic bodies in resting nuclei of *S. donnellii*, representing respectively the two arms of the X-chromosome. Our preparations, however, show clearly that there is most frequently only one such body.

It cannot be too frequently emphasized that "heteropycnosis" is a relative appearance, affected by the condition of the cell, particularly, as TINNEY (11) has shown, by the length of the interkinetic period; probably affected also by the fixation, and certainly by the staining procedure. It is possible in material destained to different degrees to find nuclei with no stain whatever, others with one, two, three, or more stained bodies, and still others whose chromosomal and nucleolar substances are uniformly and deeply stained. One expecting any particular number of heteropycnotic bodies might find evidence to fit his preconception. It is only when a very large proportion of the nuclei in satisfactorily stained material shows a like condition that significant conclusions can be drawn regarding heteropycnotic phenomena.

In median prophase stages (fig. 4), one chromosome is sometimes seen to be more condensed and much more deeply stained than the others. This condition again is characteristic of the X-chromosome at corresponding stages in *S. donnellii* and *S. texanus* (11, 12).

In lateral views of anaphases (figs. 8, 9), one chromosome, apparently one of the longer ones, is regularly seen to lag somewhat behind the others. This behavior is that typical of the X-chromosome in *S. donnellii* (2). Sometimes, also, in a polar view of an anaphase the lagging chromosome can be recognized. In figure 10 *a*, the chromosome marked X is the member of the upper anaphase group one of whose ends extends farthest downward, and the similarly indicated chromosome in figure 10 *b* is the member of the lower group which extends farthest upward. Hence, although the X-chromosome is not clearly distinguished by its size, as in the species of *Sphaerocarpos* previously studied, what may confidently be considered to

be this element is identifiable by a heteropycnotic condition in rest-
ing and prophase stages and by a delayed poleward movement in
the anaphases.

<div align="center">MALE GAMETOPHYTE</div>

The chromosome number in a male plant of *S. cristatus* is likewise
eight, as seen in late prophases (fig. 13) and in polar views of equa-
torial plates (figs. 14–17) and of anaphases (fig. 18). One of the
eight is very small, appearing either approximately spherical (figs.
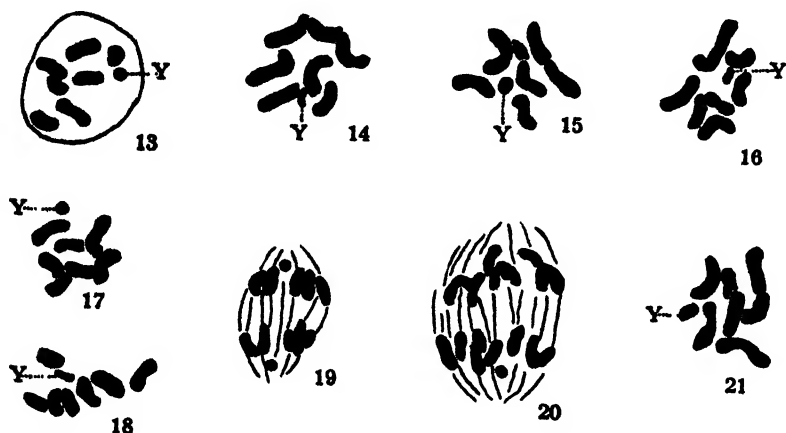
FIGS. 13–21.—Figs. 13–20, *Sphaerocarpos cristatus* ♂: fig. 13, prophase nucleus,
chromosomes organized; figs. 14–17, polar views of equatorial plates; fig. 18, anaphase
group in polar view; figs. 19, 20, anaphases seen laterally. (Figs. 13, 15, 16 from involu-
cral cells; fig. 17 from an androgonium; others from thallus cells.) Fig. 21, *S. donnellii*
♂: polar view of androgonial equatorial plate. All × c. 3600.

13, 15, 17) or somewhat elongated (figs. 14, 16, 18). Comparison
with the chromosome group of *S. donnellii* male (fig. 21) shows a
close similarity throughout. The very small chromosome present in
the male and not in the female of *S. cristatus* evidently corresponds
to the Y-chromosome of *S. donnellii*.

So far as the difficulties in the way of formulating a judgment on
this point permit, it appears that there are two relatively long
chromosomes in the male (see especially figure 16) instead of three
as in the female. This, if correct, would support the conclusion that
the X of the female gametophyte is one of the longer chromosomes.

No evidence has been found of the regular presence of a heteropycnotic body in resting or prophase nuclei of the male plant. In this respect, too, *S. cristatus* resembles *S. donnellii* as described by TINNEY (11). LORBEER's (5) statement that the Y-chromosome of *S. donnellii* is heteropycnotic is not borne out by our studies of that species.

Nor does it appear that the Y-chromosome lags behind the others during the anaphases. Sometimes, indeed, as in the anaphase shown in figure 19, it appears that the Y may precede the other chromosomes to the pole. But such appearances as that of figure 20 indicate that its behavior in this regard is variable.

While, therefore, the male chromosome complement of *S. cristatus* is closely similar to that of *S. donnellii*, and while the chromosome number is the same in both species, the marked difference in the relative size of the X-chromosome parallels the external differences which have already suggested the placing of these species in distinct subgeneric groups.

Summary

1. The chromosome number in both female and male gametophytes of *Sphaerocarpos cristatus* is eight.

2. The chromosome complement of the female includes no element that is conspicuously distinguished by its size. One chromosome (or part of one), however, is heteropycnotic in resting and prophase stages; and one chromosome lags behind the others in the anaphasic movement to the poles. These peculiarities, common to the large X-chromosome of other species of the genus, make it reasonably certain that the chromosome so distinguished (probably one of the longer ones) is the X.

3. The chromosome complement of the male includes a very small Y. This is not heteropycnotic.

4. *S. cristatus* is set apart by its external characters from other species (*S. donnellii*, *S. texanus*, and *S. michelii*) which have been studied cytologically. The much smaller size of the X-chromosome in *S. cristatus* as compared with the other species mentioned is an additional distinguishing mark.

LITERATURE CITED

1. ALLEN, C. E., A chromosome difference correlated with sex differences in *Sphaerocarpos*. Science n.s. 46:466–467. 1917.

2. ———, The basis of sex inheritance in *Sphaerocarpos*. Proc. Amer. Phil. Soc. 58:289–316. 1919.

3. HOWE, M. A., The Hepaticae and Anthocerotes of California. Mem. Torr. Bot. Club 7:1–208. 1899.

4. LORBEER, G., Untersuchungen über Reduktionsteilung und Geschlechtsbestimmung bei Lebermoosen. Zeitschr. Indukt. Abstamm. Vererb. 44:1–109. 1927.

5. ———, Die Zytologie der Lebermoose mit besonderer Berücksichtigung allgemeiner Chromosomenfragen. 1. Teil. Jahrb. Wiss. Bot. 80:565–818. 1934.

6. RICKETT, H. W., Regeneration in *Sphaerocarpos donnellii*. Bull. Torr. Bot. Club 47:347–357. 1920.

7. SCHACKE, MARTHA A., A chromosome difference between the sexes of *Sphaerocarpos texanus*. Science n.s. 49:218–219. 1919.

8. ———, A study of the chromosomes of *Sphaerocarpos texanus*. Unpublished thesis, Univ. of Wisconsin. 1919.

9. SILER, MARGARET B., Development of spore walls in *Sphaerocarpos donnellii*. BOT. GAZ. 95:563–591. 1934.

10. SMITH, F. H., The use of picric acid with the Gram stain in plant cytology. Stain Technol. 9:95–96. 1934.

11. TINNEY, F. W., Chromosome structure and behavior in *Sphaerocarpos*. Amer. Jour. Bot. 22:543–558. 1935.

12. ———, Chromosome structure and behavior in *Sphaerocarpos texanus*. Amer. Jour. Bot. 22:699–704. 1935.

13. WOLFSON, A. M., The chromosomes of *Sphaerocarpos texanus*. Amer. Jour. Bot. 14:516–519. 1927.

EFFECT OF AUXINS FROM SOME GREEN ALGAE
UPON PHYTOPHTHORA·CACTORUM

LEON H. LEONIAN[1]

(WITH TWO FIGURES)

There are many references in the literature concerning the auxins and hormones produced by bacteria, fungi, and phanerogams. In so far as the writer knows, however, no work has been done with algae.

Through the courtesy of Dr. FLORENCE E. MEIER, four pure cultures of unicellular green algae were obtained as follows, *Chlorella viscosa, Coccomyxa simplex, Oocystis naegelii*, and *Scenedesmus flavescens*. Originally these cultures were secured with the specific purpose of testing upon them the growth-promoting substances extracted from mature garden peas. These substances are a controlling factor in the growth of a great many filamentous fungi, while in case of others they produce a remarkable stimulation of both growth and reproduction.

All four of these algae make a moderately good growth on a medium consisting of 5 gm. of dextrose, 1 gm. of potassium nitrate, 0.5 gm. of dihydrogen potassium phosphate, 0.25 gm. of magnesium sulphate, and 20 gm. of agar-agar in 1000 cc. of distilled water. When, however, 0.05 per cent of pea extract (obtained by extracting ground peas with 95 per cent of alcohol in soxhlet) was added to this solution, the algal colonies grew much more rapidly (fig. 1). Ash of garden peas exerted no such effect. *Chlorella viscosa* and *Coccomyxa simplex* eventually lose their bright green color on agar cultures containing no pea extract and become pale yellow, with the green color confined to a small area around the original inoculum. Under the microscope the cells appear almost colorless and their protoplasm seems granular and thin. In cultures containing the pea extract, however, the colonies retain their color throughout their life (five months), and the individual cells appear bright green, plump, and

full of protoplasm. While in case of *Ooocystis naegelii* and *Scendesmus flavescens* there is some stimulation of growth and especially of color, it is not so pronounced.   ·

When transferred to the nutrient solution of the foregoing composition, *Phytophthora cactorum* fails to make any growth whatever, while excellent growth follows upon the addition of a trace of pea extract. Since all four of the algae can grow in this solution, one may safely assume that they are capable of manufacturing their own growth-promoting substances. Yet the slower growth and the loss of

FIG 1 —Effect of growth-promoting substances upon *Oocystis naegelii* Left (check), no growth-promoting substances present in the nutrient agar Right, a trace of growth-promoting substances from the garden peas added to the agar

color seem to show that the growth-promoting substances manufactured by the algae themselves, and those extracted from garden peas, may either be unlike chemically or may differ quantitatively.

The next procedure was to test the effect of the algal substances upon the development of *Phytophthora cactorum*. The following method was used: The algae were grown in flasks containing 200 cc. of the nutrient solution already mentioned but having received no pea extract. After 30 days at room temperature and in diffused light these pure cultures were filtered aseptically through two thicknesses of sterilized, fine filter paper. Ten cc. of the filtrate was poured into preparation dishes of 25 cc. capacity and inoculated with *P. cactorum*. The check solution in which no algae had been grown was treated similarly. There was no growth in the check, while excellent growth resulted in the filtrates of the cultures of *Coccomyxa simplex*

FIG. 2.—Effect of growth-promoting substances from some unicellular algae upon the growth of *Phytophthora cactorum*. 1 (check), no algae grown in the nutrient solution; 2, filtrate of *Oocystis naegelii*; 3, filtrate of *Coccomyxa simplex*; 4, filtrate of *Chlorella viscosa*; 5, filtrate of *Scenedesmus flavescens*.

and *Oocystis naegelii;* the filtrates of *Chlorella viscosa* and *Scenedesmus flavescens* induced only a fair growth (fig. 2).

In addition to auxin content, the algal filtrates were tested also for sexuality-promoting substances. *Phytophthora cactorum* produces an abundance of oogonia and antheridia on suitable media, while no such bodies are formed on others. If 0.2 per cent of proteose peptone be added to the foregoing nutrient solution, *P. cactorum* will grow very well, but without forming any reproduction bodies. Thus it is possible to obtain a well nourished but sterile mycelium which can be subjected to the action of various environmental factors. Four days after the transfer of the inoculum, the fungus produces a colony large enough to be washed in distilled water and be transferred to any de-

TABLE I

EFFECT OF ALGAL FILTRATES UPON P. CACTORUM

FILTRATE	SPORANGIA	OOGONIA
Coccomyxa simplex................	Abundant	Abundant
Oocystis naegelii..................	Abundant	Abundant
Chlorella viscosa..................	Very abundant	Few
Scenedesmus flavescens...........	Abundant	Few
Check (nutrient solution).........	None*	None

* If allowed to remain in the nutrient solution eight days, the mycelium forms sporangia but no oogonia.

sired solution. The mycelial colonies of *P. cactorum* were washed thoroughly in sterile distilled water and then transferred to a small amount of the algal filtrate (2 cc. in preparation dishes of 25 cc. capacity); when after three days at 20° C. they were examined under the microscope, it was observed that both sporangia and oogonia had formed (table I).

In connection with our project on the isolation and identification of growth and sexuality promoting substances, Dr. VIRGIL G. LILLY and the writer have tested a great many substances, green algae included. *Oocystis naegelii* was first grown in a solution consisting of the essential salts and glucose; a month later the algal cells were filtered, washed with distilled water and extracted. The alcoholic extract proved to be rich in both growth and sexuality promoting factors for *Phytophthora cactorum,* while the ether extract failed to

induce any growth but was found to be very rich in sexuality pro-
moting factors. This is in general agreement with results which we
have obtained from other substances.

As the extracts from the algal cells proved to be much richer than
the filtrate in their ability to induce growth and sexual reproduction,
it follows that the factors responsible for this are manufactured by
the algae themselves instead of being due to any chemical changes
brought about in the medium by the growing cells of *Oocystis
naegelii*.

It is comparatively safe to conclude that these algae produce sexu-
ality-promoting substances in addition to auxins, and that the quan-
tity of these substances diffused into the solution varies with differ-
ent species of algae in so far as the response of *P. cactorum* is con-
cerned. Whether these substances are diffused through the plasma
membrane of the living algal cells or whether they are liberated upon
the death and subsequent lysis of the cell is not yet clear. The writer
(2) has demonstrated that uninjured roots of corn seedlings do not
give off auxins into the solution in which the roots are kept growing,
while from the proximal part of a detached root considerable quanti-
ties of auxins diffuse out into the medium. Filamentous fungi, on
the other hand, give off auxins into the medium without having their
mycelium subjected to any apparent injury. Presumably the auxins
diffuse through the uninjured plasma membrane. An extensive series
of dialysis experiments by the writer and by his collaborator, Dr.
LILLY, have shown that auxins from various sources readily pass
through parchment paper and through collodion sacs. Furthermore,
it is well known that certain pathogenic bacteria such as *Hemophilus
influenzae*, which grow in artificial cultures with great difficulty, pro-
duce a luxuriant growth in the immediate neighborhood of a colony
of some staphylococcus because of the growth-promoting substances
which diffuse into the agar from the colony of the latter organism.
Such data tend to indicate that at least in certain cryptogamic organ-
isms the auxins may diffuse out into the medium through unin-
jured plasma membrane.

The foregoing results show that the algal and fungal flora of the
soil may possibly exert some influence upon green plants as well as
upon cryptogamic inhabitants of the soil. ITO and SHIMADA (1) and

SHIMADA (3) have shown that the addition of a filtrate of *Gibberella fujikuroi* to nutrient solutions induces a remarkable stimulation of growth in seedlings of rice, wheat, barley, Indian corn, Azuki bean, and soy bean. This stimulation is apparently caused by the auxins given off by the fungus. It is possible that similar results may occur under field conditions by the association of suitable cryptogamic flora and phanerogamic plants.

The data obtained in this work also shed some light upon the relationship between the fungi and algae which grow together to form lichens. It is probable that the lichen fungi utilize not only carbohydrates furnished by the algae, but growth substances as well, and that their compatibility and the subsequent associations may not be controlled so much by the nature of carbohydrates as by the nature, the quantity, and the availability of the growth- and reproduction-inducing substances.

Summary

1. Four pure cultures of unicellular green algae, *Chlorella viscosa*, *Coccomyxa simplex*, *Oocystis naegelii*, and *Scenedesmus flavescens*, have been used. Especially the first two show a remarkable stimulation under the action of growth-promoting substances extracted from garden peas. These algae also retain their green color in the presence of these substances, while in their absence the color largely fades into a yellowish tone.

2. All four of the algae form both growth and sexuality-promoting factors for *Phytophthora cactorum*.

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LITERATURE CITED

1. ITO, SEIYA, and SHIMADA, SHOICHI, On the nature of growth-promoting substances excreted by "Bakanae" fungus. Ann. Phytopath. Soc. Japan 2:322–338. 1931.

2. LEONIAN, LEON H., The effect of auxins upon *Phytophthora cactorum*. Jour. Agr. Res. 51:277–286. 1935.

3. SHIMADA, SHOICHI, Further studies on the nature of the growth-promoting substance excreted by the "Bakanae" fungus. Ann. Phytopath. Soc. Japan 2:1–11. 1932.

CHROMOSOME NUMBERS AND ELECTROPHORESIS
OF LATEX IN ASCLEPIAS

LAURENCE S. MOYER[1]

(WITH SEVEN FIGURES)

Introduction

Evidence has recently been presented (8) to show that the surfaces of the latex particles of *Euphorbia* species are constant in composition, depending, within broad limits, only on the species and not upon the environment. This specific character of the surfaces is exhibited in the isoelectric points, wetting properties (10), and the shapes of the electrophoretic mobility curves when plotted against pH (6). When these mobility curves were classified according to their shapes, they fell into groups in correspondence with the systematic groupings of the species. Furthermore, in the section Poinsettia, the presence of an aberrant member, *Euphorbia heterophylla*, was first indicated by its latex behavior and then was shown to be a polyploid from chromosome counts (7). All these observations were confined to a single genus. To test the generality of these phenomena, determinations of chromosome numbers and latex mobility curves have been made on species of *Asclepias*.

The investigations were limited by the paucity of latex in certain species and by the number of available species growing within convenient reach of the laboratory. Although the number of species reported here is not so great as could have been desired, enough data are available to demonstrate that the use of latex as a taxonomic tool need not be limited to *Euphorbia*.

Methods

The technique of the chromosome counts was the same as previously described (7). All root tips were cut at 8μ. For the electrophoretic measurements, latex was in all cases suspended in the buffer solutions in the field or greenhouse; all determinations were performed within an hour after suspension, usually within ten minutes. Acetic acid-sodium acetate buffers of constant ionic strength ($\mu =$

[1] National Research Fellow in Botany.

1/50) were used. The specific resistivity of these buffers is nearly
constant, making them superior to mixtures of constant molarity (as
used before, 6) for electrophoretic work. The preparation of such
buffers has been described (9). The technique of electrophoresis was
the same as before (6, 9). Mobilities are given in μ/sec./volt./cm.,
corrected to 25° C.
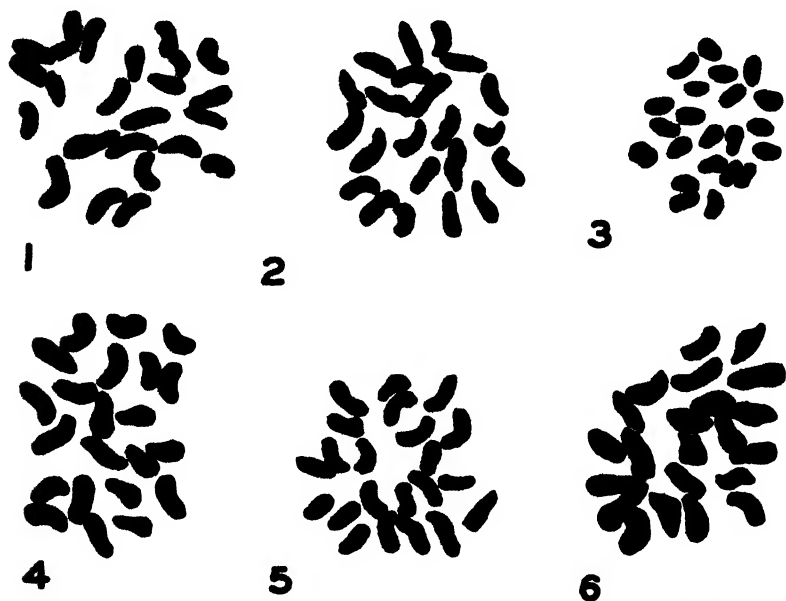
FIGS. 1–6.—Somatic metaphase plates of *Asclepias:* fig. 1, *A. syriaca* 2n = 24; fig. 2,
A. incarnata 2n = 22; fig. 3, *A. curassavica* 2n = 22; fig. 4, *A. salicifolia* 2n = 22; fig. 5,
A. latifolia 2n = 22; fig. 6, *A. tuberosa* var. *sulfurea* 2n = 22. ×4000.

Results

The results of the chromosome count are depicted in figures 1–6.
Asclepias curassavica L., *A. salicifolia* Lodd., *A. incarnata* L., *A.
latifolia* Rafin., and *A. tuberosa* var. *sulfurea* L. have 22 somatic
chromosomes whereas *A. syriaca* L. (= *A. cornuti* Decaisne) has 24.[2]
The morphology of the chromosomes is noticeably different among
the different species although the chromosomes are small and short.

The electrophoretic curves of the latex particles are shown in
figure 7. The latex particles seem slightly larger than those of

[2] These counts have been checked by Mr. A. O. DAHL in addition to the writer.

Euphorbia and exhibit more tendency to cohere. The chromosome numbers and isoelectric points are summarized in table I. No electrophoretic data on *A. latifolia* or *A. tuberosa* var. *sulfurea* are avail-
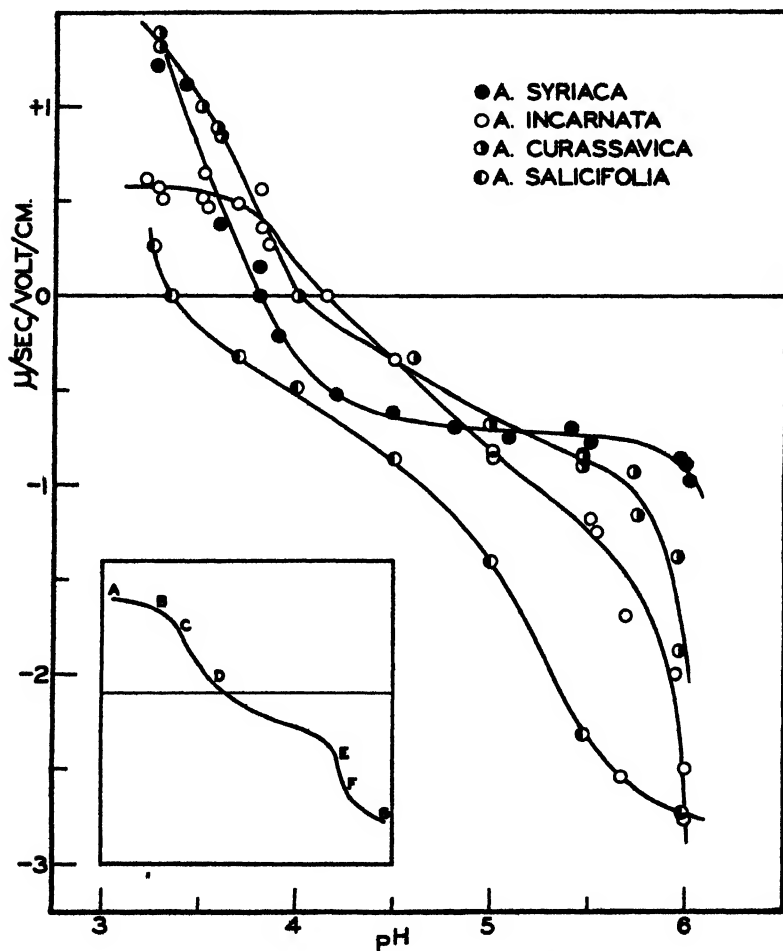
FIG. 7.—Electrophoretic mobility curves of latex particles from species of *Asclepias*. Inset shows hypothetical curve (over a wide pH range) approximated by the various species.

able, owing to the scarcity of latex in these species. As may be seen, the extra chromosome of *A. syriaca* causes no sharp change in its isoelectric point or curve shape from the rest. The isoelectric points and

shapes of the mobility curves (fig. 7) of *A. syriaca, A. incarnata,* and
A. curassavica over the range from pH 3 to 6 are rather closely allied.
E. salicifolia is the most divergent species of the lot. Results were
sometimes more variable than those obtained with *Euphorbia* but
with proper care data could be checked. The present isoelectric pH
values for *A. syriaca* and *A. curassavica* agree well with results (pH
3.9 and 4.1 respectively) obtained under different conditions with
plants from a different source (10). This slight divergence of 0.1 pH
unit may be ascribed to differences in the concentrations of the

TABLE I

CHARACTERISTICS OF ASCLEPIAS SPECIES

SPECIES	LATEX ISO-ELECTRIC POINT (pH)	CHROMO-SOME NUMBER (2n)	POSITION ON HYPOTHETI-CAL CURVE
A. incarnata.............	4.1	22	A–F
A. curassavica...........	4.0	22	B–F
A. syriaca...............	3.8	24	C–E
A. salicifolia.............	3.4	22	D–G
A. latifolia..............	....	22	....
A. tuberosa sulfurea.......	....	22	....

buffer systems. Plants tapped after fruiting sometimes yielded di-
vergent and variable results; such plants were not in active growth
and appeared much darker in leaf color. Only data from young
healthy plants are presented here.

Discussion

These curves are limited in extent by the range of the buffer (pH3–
6). Introduction of another buffer introduces complications which
would invalidate comparisons. Since the range is so narrow, only a
part of the total curve for each species is represented. From this,
however, we can construct a hypothetical curve with which all of the
species here reported agree in shape for the portion over which they
extend. The portions of the hypothetical curve fitted by the various
species are shown in table I and figure 7 (inset). It will be noted that
as the isoelectric point descends on the pH scale the curve is merely
moved over one more letter in each species. Hence, the curve of each
species would represent the portion of this hypothetical curve (shift-
ing with the isoelectric point) confined between the limits of the

measurements. Minor differences in shape do occur but they seem to be secondary in nature.

By the use of the Mudd interfacial technique it was shown (10) that *A. syriaca* and *A. curassavica* possess a hydrophilic surface which strongly resists wetting by oil. The position of the isoelectric points and the shapes of the mobility curves for these species indicate that proteins are present on the latex particle surfaces (1), in complete agreement with the wetting data. The surfaces are undoubtedly complex in nature. No wetting determinations have been made on *A. salicifolia* and *A. incarnata* latex.

STEVENS (11), GAGER (5), and FINN (2) also report 24 chromosomes as the diploid number for *A. syriaca*. FRYE (3, 4) states that the haploid counts for *A. tuberosa* L. and *A. sullivantii* Engelm. are about 5. He also states that gametophytic material of *A. verticillata* shows about 8 chromosomes but he was unable to give more definite figures. No other species of *Asclepias* appear to have been counted. The lack of a marked variation in the shape of the curve of the aneuploid species (*A. syriaca*) indicates that genes controlling the surfaces of its latex particles are not located in the extra chromosome.

Since so few species have been investigated, an attempt to show any sort of phylogenetic arrangement on the basis of either chromosomes or latex curves would seem premature. The data indicate, however, that species relationships are shown by the electrophoretic curves of the latex particles of *Asclepias* as well as in the case of *Euphorbia*.

Summary

1. The electrophoretic mobility curves for latex particles of *Asclepias syriaca*, *A. curassavica*, *A. incarnata*, and *A. salicifolia* have been determined.

2. The chromosomes from the root tip cells of these species and also *A. tuberosa* var. *sulfurea* and *A. latifolia* have been counted. *A. syriaca* has 24 somatic chromosomes while the rest of the species have 22.

3. The presence of this extra chromosome in *A. syriaca* is not shown by the electrophoretic curve, indicating that genes for the latex particle surface are not located in this chromosome.

4. The isoelectric points of *Asclepias* appear to be constant and

dependent only on the species, within broad environmental conditions.

5. The surfaces of the latex particles are, at least in part, coated with proteins.

6. Species relationships are shown by the electrophoretic curves and isoelectric points of the latex particles of *Asclepias* as well as of *Euphorbia*. All of the species in the present work are characterized by the same type of mobility curve.

The writer wishes to thank the Botany Department of this University for extending the facilities of its equipment during the preparation of the cytological material. Thanks are especially due to Professor R. A. GORTNER, in whose laboratory the investigations were performed, for his kind help and suggestions.

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LITERATURE CITED

1. ABRAMSON, H. A., Electrokinetic phenomena. New York. 1934.
2. FINN, W. W., Male cells in angiosperms. I. Spermatogenesis and fertilization in *Asclepias cornuti*. BOT. GAZ. 80:1–25. 1925.
3. FRYE, T. C., Development of the pollen in some Asclepiadaceae. BOT. GAZ. 32:325–331. 1901.
4. ———, A morphological study of certain Asclepiadaceae. BOT. GAZ. 34:389–413. 1902.
5. GAGER, C. S., The development of the pollinium and sperm cells in *Asclepias cornuti* Decaisne. Ann. Bot. 16:123–148. 1902.
6. MOYER, L. S., Species relationships in *Euphorbia* as shown by the electrophoresis of latex. Amer. Jour. Bot. 21:293–313. 1934.
7. ———, Electrophoresis of latex and chromosome numbers of poinsettias. BOT. GAZ. 95:678–685. 1934.
8. ———, The constancy of latex isoelectric points. Protoplasma 21:588–593. 1934.
9. ———, Untersuchungen über die Elektrophorese der Cholesterinsuspensionen. Biochem. Zeitschr. 273:122–131. 1934.
10. ———, On the surface composition of certain latex particles. Amer. Jour. Bot. 22:609–627. 1935.
11. STEVENS, W. C., The behavior of kinoplasm and nucleolus in the division of the pollen mother cells of *Asclepias cornuti*. Kansas Univ. Quart. 7:77–85. 1898.

CURRENT LITERATURE

Invisible Radiations of Organisms. By OTTO RAHN. Berlin: Gebrüder Born-
traeger, 1936. Pp. x+215.

About 14 years ago GURWITSCH published his first papers dealing with the
causes of cell division, and the existence of radiations emanating from the
rapidly dividing cells of root tips, which radiations excite other root tips to in-
creased cell division, or excite yeast cells to more rapid budding. These phe-
nomena soon came to be known as mitogenetic radiations. In the period fol-
lowing these original contributions of GURWITSCH, many investigators have
worked in this field and there has been controversy as to the reality of the
mitogenetic radiation phenomena. Many European investigators have sup-
ported GURWITSCH's observations and interpretations, while some of the
American workers have been conservative and skeptical.

The results of the work done since 1922 are brought together in summary
fashion as a monograph (Protoplasma Monographien, vol. IX), by OTTO
RAHN, Cornell bacteriologist, who believes in the reality of the phenomena, al-
though it appears that he has had rather moderate experience in detecting
them in his own work. In the foreword he claims that invisible radiations from
organisms are not at all strange, and that if GURWITSCH had not discovered
them some years ago, they would now be predicted from the results of physico-
chemical investigations.

The introductory chapters, contributed by SIDNEY W. BARNES, will be
valuable to students of biology because they contain a clear and concise explana-
tion of radiation physics, wave theory, quantum theory, dispersion, intensity
measurements of visible and invisible radiations, physical, chemical, and second-
ary sources of radiation, and the effects of invisible radiations upon chemical
reactions and upon living cells.

The mitogenetic radiations are given extensive treatment in chapters IV–VI.
Chapter IV considers the methods used for detection of biological radiation;
chapter V discusses the special characteristics of these radiations; and chapter
VI makes an analysis of the mitogenetic effect.

The last chapter is devoted to the significance of biological radiations in
biology, medicine, and agriculture. In connection with blood and carcinoma
radiations, medicine may find considerable aid in diagnostic and other problems.
At the close of chapter VII the author presents the "outlook" for this field, and
also gives a brief summarizing statement of the various chapters. There are
author and subject indices, the former incomplete but extensive enough for the
purposes of the monograph. The author is convinced from his own experiences

as well as from a study of the literature that mitogenetic radiation is a fact. He has undoubtedly presented the subject in its best light. If the skeptics and the enthusiasts could work together for a while the reasons for controversy would probably soon be eliminated. RAHN claims that errors and misunderstandings rest on both sides, and he has tried to point out some of the sources of disagreements. It does not seem unreasonable to ask those who believe in mitogenetic phenomena to define their methods and conditions clearly enough that the skeptically inclined may follow them. One of the difficulties seems to be that failures to obtain the phenomena are explained away by the believers as due to the use of material in the "wrong physiological state," or by saying that "unknown factors" have interfered. The monograph is interestingly written, and will prove valuable in stimulating work toward the settlement of the controversies in this field.— C. A. SHULL.

The Theoretical Significance of Vernalization. By N. A. MAXIMOV. Imp. Bur. Plant Genetics, Herb. Publ. Series Bull. 16, 1934. Pp. 14.

Vernalization and Phasic Development of Plants. Imp. Bur. Plant Genetics Bull. 17, 1935. Pp. 155.

Initiated as a practical agricultural method of accelerating the development of economic plants and solving such problems as the transformation of winter wheat into spring wheat, vernalization has involved such a control of the phenomena of growth and development of living organisms that it has attracted the attention of plant physiologists throughout the world. Since much of the experimental work in this field has been carried on by Russian scientists, among whom LYSENKO has been a leader, it is fitting that its theoretical significance should be discussed by a leading Russian plant physiologist. MAXIMOV has pointed out that much of the recent investigation is a continuation and extension of the work of KLEBS, who proved that the life processes of plants, including reproduction, are to a great extent subject to the control of external conditions, and that by modifying these conditions man may change the internal state of many plants and so alter such phases of development as flowering and seed production.

Following to a large extent similar principles, LYSENKO's hypothesis of vernalization is largely based on the method of subjecting slowly germinating seeds of winter wheat to a temperature little above 0° C. for periods of 10 to 60 days. The seed is then sown in suitable soil, at normal spring temperatures, and the resulting plants behave like spring wheat and produce a crop during the same summer. His theoretical concepts are that growth and development are not identical phenomena, that the entire process of the development of an annual seed plant consists of individual steps or stages, that these stages always proceed in a strict sequence and a subsequent stage cannot set in until the preceding stage has been completed, and that different stages of the same plant require different external conditions for their completion.

These concepts MAXIMOV has examined in detail and discussed their strong and weak points. He regards the hypotheses as well founded but probably subject to modification upon further experimentation.

In a more recent bulletin from the same source, an elaboration of LYSENKO's theories is presented, together with summaries of the results obtained by many other scientists who have investigated vernalization in a considerable number of plants. The idea that development consists of a series of stages has been elaborated and the "thermo-stage" and the "photo-stage" are discussed, the latter involving a somewhat different interpretation of "short-day plants." A third stage is less clearly differentiated and modifications of LYSENKO's theory by other investigators are given. It is evident that vernalization and its modifications have been extensively investigated in the Soviet Union. As the results of these investigations have been published almost exclusively in Russian, the Imperial Bureaux of Plant Genetics has rendered the scientists of Great Britain and America a great service by making the results available in English. The investigations of the problems related to vernalization in other countries have been summarized and brought down to date and a bibliography of nearly one hundred titles is appended.—G. D. FULLER.

The Garden Encyclopedia. Edited by E. L. D. SEYMOUR. New York: Wm. H. Wise & Co., 1936. Pp. x+1300. Illustrated.

Although rather brief encyclopedias on horticulture are already available, this newcomer should find a place among the books of everyone interested in that subject. The variety of subjects treated is widely comprehensive, but each is discussed in sufficient detail really to be useful to the reader. The botanical information given is accurate, concise, and helpful, but with no attempt at being exhaustive. The horticultural suggestions and directions are clear cut and as definite as can be expected, considering the wide variety of conditions under which various forms may be grown. The text is well illustrated with halftones and effective line drawings. Tabular summaries on some subjects are very useful.—E. J. KRAUS.

The Biochemistry of the Lipids. By HENRY B. BULL. Division of Biochemistry, University Farm, St. Paul, Minn.

In this mimeographed text the chemistry of the important groups of fatty acids, soaps, alcohols, sterols, fats, oils, phospholipids, and glycolipids has been ably summarized and excellently organized into a readable text. Tables of properties, graphs, and graphic formulae have been used so freely that the book is valuable for reference to this insufficiently known group of substances. Numerous references to the physiology of the lipids should be especially valuable to the plant physiologist.—R. B. HARVEY.

GENERAL INDEX

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Psaronius sp.

A badly crushed stem, polystichous type, with numerous steles and bands of sclerenchyma. It is too much crushed to permit of specific determination.

Heterangium tiliaeoides Will.

Several stems were identified.

Medullosaceae

A portion of a stem, obviously belonging to the family Medullosaceae, but too incomplete for even generic determination, and four species of petioles of *Myeloxylon*, were noted.

Summary

1. This paper records the occurrence of a number of plants found as petrifacts from Calhoun coal mine, Richland County, Illinois. The plants belong to the genera *Calamites, Sphenophyllum, Lepidodendron, Lepidostrobus, Sigillaria, Mazocarpon, Botryopteris, Anachoropteris, Psaronius, Heterangium, Myeloxylon,* and *Cardiocarpus*.

2. It appears that there are no constant anatomical distinctions between the Eu-Sigillariae and the Sub-Sigillariae.

3. The branching of petioles of *Botryopteris americana* is described.

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LITERATURE CITED

1. ARBER, E. A. N., and THOMAS, H. H., On the structure of *Sigillaria scutellata* Brongn. and other Eu-Sigillarian stems in comparison with those of other Palaeozoic lycopods. Phil. Trans. Roy. Soc. London 200:133–166. 1008.

2. BENSON, MARGARET, New observations on *Botryopteris antiqua* Kidston. Ann. Bot. **25**:1045–1057. 1911.

3. ————, *Mazocarpon* or the structural *Sigillariostrobus*. Ann. Bot. **32**:569–589. 1918.

4. BERTRAND, C. E., and CORNAILLE, F., Les caractéristiques de la trace foilaire botryoptéridienne. Compt. Rend. Acad. Sci. Paris **150**:1019. 1910.

5. BERTRAND, P., L'étude anatomique des fougères anciennes et les problèmes qu'elle soulève. Progressus Rei Botanicae. IV. 1911.

6. ————, Observations sur la classification des fougères anciennes. Bull. Soc. Bot. France **80**:527. 1933.

7. BRONGNIART, A., Recherches sur les graines fossiles. Paris. 1881.

8. FONTAINE, W. M., and WHITE, I. C., The Permian or Upper Carboniferous flora of West Virginia and S.W. Pennsylvania. p. 96. 1880.

9. HOVELACQUE, M., Recherches sur le *Lepidodendron selaginoides* Sternb. Mém. Soc. Linnéenne de Normandie. Vol. XVII. No. 1. 1892.

10. KIDSTON, R., On the internal structure of *Sigillaria elegans* of BRONGNIART'S Histoire des végétaux fossiles. Trans. Roy. Soc. Edinburgh **41**:533–550. 1905.

11. RENAULT, B., Étude du genre *Botryopteris*. Ann. Sci. Nat. 6th ser. **1**:220–240. 1875.

12. ————, Flore fossile du bassin houiller d'Autun et d'Épinac. pp. 27–30; 47–59. 1893.

13. SEWARD, A. C., A Persian *Sigillaria*. Phil. Trans. Roy. Soc. London **221**:377–390. 1932.

14. WATSON, D. M. S., On a confusion of two species (*Lepidodendron Harcourtii* Witham and *L. Hickii* sp. nov.) under *Lepidodendron Harcourtii* Witham in WILLIAMSON'S XIX Memoir, with a description of *L. Hickii*, sp. nov. Mem. Proc. Manchester Phil. Soc. 51. Pt. 13. 1907.

15. ZEILLER, R., Revue des travaux de paléontologie végétale. Rev. Gén. Bot. **9**:pl. 20, fig. 3. 1897.

CYTOLOGICAL STUDIES IN THE CHENOPODIACEAE

I MICROSPOROGENESIS AND POLLEN DEVELOPMENT

George Olds Cooper

(WITH FORTY-TWO FIGURES)

Introduction

The purpose of this investigation was to describe in detail the male gametes and to determine the chromosome numbers in certain members of the Chenopodiaceae. Most observers working with angiosperms have mentioned male nuclei as occurring in the micro-gametophyte, but in only a few instances have the male gametes been described as distinct cells. Tuschnjakowa shows them to be cells in *Spinacea oleracea* (10, fig. 20a). The presence of such male cells, aside from those in the Chenopodiaceae, has been observed by Frye (5) and Finn (4) in certain members of the Asclepiadaceae, by Wylie (12) in *Elodea canadensis* and (13) in *Vallisneria spiralis*, by Shattuck (8) in *Ulmus americana*, by Poddubnaja-Arnoldi (6) in *Scabiosa purpurea*, and by Cooper (3) in *Portulaca oleracea*. Stomps (9), Winge (11), Artschwager (1), Tuschnjakowa (10), and Billings (2) have described microsporogenesis or have recorded the chromosome numbers in some members of the Chenopodiaceae.

Materials and methods

Floral tips of *Kochia trichophylla* Staph., *K. scoparia* Schrad., *Chenopodium hybridum* L., *C. album* L., *Atriplex patula* var. *hastata* L., and *Salsola kali* L. were collected in the summer of 1933 on or near the campus of the University of Wisconsin by Dr. D. C. Cooper, Dr. K. L. Mahony, and the writer. The identification of the species was verified by Dr. J. J. Davis. The material was fixed in Carnoy's solution, imbedded in paraffin, and sectioned at a thickness of 14 μ. The sections were stained in Delafield's haematoxylin because of its clear differentiation of the parts of the mature pollen grain.

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All drawings were made with an Abbé camera lucida at table level. A no. 4 Leitz ocular and an achromatic oil immersion N.A. 1.32 objective were used.

Investigation

Kochia trichophylla

The stamen contains rows of primary sporogenous cells which divide to form microspore mother cells (fig. 1). These cells are larger than the surrounding cells of the theca. During the maturation of the spore mother cells the nuclei of the tapetal cells undergo mitosis; ultimately each tapetal cell has two nuclei.

At diakinesis nine pairs of chromosomes are present in a spore mother cell (fig. 2). The paired chromosomes vary as to shape; one pair, larger than the others, appears to be attached to the nucleolus. The cell enlarges markedly during heterotypic prophases.

Figure 3 shows a polar view of nine chromosomes on the equatorial plate. Although the several pairs of chromosomes varied in size during diakinesis, they appear very similar in size on the heterotypic spindle. This spindle (fig. 4) is surrounded by a dense cytoplasmic zone. Near the periphery of the cell is a granular zone. These two zones are separated by a region containing vacuoles.

The spindles of the homoeotypic division lie side by side, either parallel to or at right angles to each other. Each spindle is surrounded by a dense cytoplasmic zone similar to that found in the heterotypic division. That region of the two cytoplasmic zones which lies between the spindles is particularly dense at the anaphase stages of nuclear division (fig. 5).

The position of the four nuclei after the completion of the homoeotypic division suggests that the homoeotypic spindles may often lie at right angles to each other (fig. 6). The nuclei are connected by the two prominent homoeotypic spindles and four less prominent spindles which are perhaps the remains of the heterotypic spindle. Thickenings appear on the fibers of each of these spindles midway between each pair of nuclei.

After the microspores have become separated they enlarge and their walls thicken, taking on the markings characteristic of the pollen grains of the Chenopodiaceae. The nucleus enlarges and

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